Development of a cost-effective system for ovoviviparous production of *Artemia* nauplii at low-salinity as live food for the larvae of the African catfish (*Clarias gariepinus:* Burchell, 1822)

by

Richard Bwala

Dissertation presented for the joint degree of
Doctor of Philosophy Agricultural Sciences
Doctor of Applied Biological Sciences: Aquaculture

at

Stellenbosch University[1] & Ghent University[2]
[1]Animal Sciences Department, Faculty of AgriSciences
[2]Laboratory of Aquaculture & *Artemia* Reference Center, Faculty of Bioscience Engineering

Supervisor: Dr Khalid Salie (Stellenbosch University, South Africa)
Supervisor: Prof Dr Gilbert Van Stappen (Ghent University, Belgium)
April, 2019

Afrikaans translation of the title:

Ontwikkeling van ‘n koste-effektiewe sisteem vir die ovovivipariese lae-saliniteit produksie van Artemia nauplii as lewendige voer vir larwes van die Afrika katvis (Clarias gariepinus: Burchell, 1822)

Dutch translation of the title:

Ontwikkeling van een kost-effectief systeem voor de ovovivipare productie bij laag zoutgehalte van *Artemia*-nauplii als levend voedsel voor de larven van de Afrikaanse katvis (*Clarias gariepinus*: Burchell, 1822)

This study was funded by the West African Agricultural Productivity Programme (WAAPP-Nigeria) through a PhD scholarship to the author

Dean, faculty of AgriSciences, Stellenbosch University: Prof Danie Brink

Rector, Stellenbosch University: Prof Wim de Villiers

Dean, faculty of Bioscience Engineering, Ghent University: Prof dr ir Marc Van Meirvenne

Rector, Ghent University: Prof dr ir Rik Van de Walle

Members of the Examination and Reading Committee:

Dr Elsje Pieterse
Department of Animal Sciences, Stellenbosch University, South Africa
elsjep@sun.ac.za

Em Prof dr Patrick Sorgeloos
Laboratory of Aquaculture & *Artemia* Reference Center, Ghent University, Belgium
patrick.sorgeloos@ugent.be

Prof Cliff Jones
Department of Ichthyology and Fisheries Science, Rhodes University, South Africa
c.jones@ru.ac.za

Dr Betty Nyonje
Kenya Marine & Fisheries Research Institute, Mombasa, Kenya
bnyonje@hotmail.com

DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated) that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification. This dissertation has also been presented at Ghent University, Belgium in terms of a joint-degree agreement.

Date: April, 2019

SUMMARY

The brine shrimp *Artemia*is a small crustacean used as live food in fish and shell fish culture. Among other live food types which are commonly used in aquaculture, *Artemia* is widely known and accepted mainly due to the ease with which it can be used in hatcheries either in the form of nauplii, hatched from cysts, or as decapsulated cysts which are sold as off-the-shelf products. Supply of cysts has been hampered due to discontinuity of *Artemia* distribution in natural biotopes, meteorological fluctuations, climatological changes and possible overharvesting of resources. This has led to instability and resulted in fluctuations and sometimes reduction ofthe global production. Hence regional cyst supply has been insured in many places through artificial inoculation and man-managed production in salt ponds. Hatcheries in sub-Saharan Africa have so far relied on importation of product of variable quality, thereby adversely affecting fish production efficiency and reducing the competitiveness of aquaculture in this region.

This research work contributes to the knowledge on the alternative ways for the supply of *Artemia* as live food for use in hatcheries. Firstly, literature was reviewed focusing on the general overview of the status of aquaculture globally and within the sub-Saharan region of Africa; the taxonomy, biology, culture and applications of *Artemia*; on the taxonomy, biology and aquaculture production of the African catfish *Clarias gariepinus*, and the requirement of live food for their larvae. Among other challenges which aquaculture faces in Africa, a common re-occurring factor identified is the insufficiency or non-availability of fish seed which in turn has been partly associated to the scarcity of live food which is an essential component in the hatchery production of the larvae of many cultured fish species.

Secondly, data are presented on a study of low salinity (0 – 32 g L^{-1}) culture of *Artemia* using four strains from two bisexual [Great Salt Lake (GSL), Vinh Chau (VC)] and two parthenogenetic [Tuz (TUZ), Balikun (BLK)] populations. Laboratory tests were performed, firstly, to measure axenically the survival of instar I and II at low salinity during a 48 h period. The use of the two instars was based on the hypothesis that ambient salinity does not affect the embryo and instar I nauplii of *Artemia*, and to assess if using instar II nauplii to inoculate culture would affect survival. Similarly, survival of the *Artemia* at two inoculation ages (i.e. nauplii and pre-adult) was tested xenically over a nine days period at these low salinities. The two inoculation ages were used based on the hypothesis that mortality rate usually reduces as *Artemia* attains maturity. Results of both tests showed that the low salinity influenced survival with respect to the strain types. With respect to the two instar developmental stages, no difference was observed as a result of the low salinities tested. With regard to the inoculation ages, no differences were also observed among the salinities tested, except atlower (5 g L^{-1}) salinity where the lowest survival was recorded. Furthermore, a test on the effect of low salinity on the reproductive and life traits showed that both salinity and strain

had an influence on the parameters measured. Generally, high survival was observed at those low salinities where survival occurred, but the highest was recorded at 20 and 32 g L^{-1} (control) salinity. Overall the best performance in terms of the reproductive and life traits measured was observed with the Great Salt Lake (GSL) strain. In particular, the GSL strain had the highest total offspring as well as the number of offspring produced as nauplii. We conclude that 20 g L^{-1} salinity and the GSL strain are the most suitable for use in mass culture.

Subsequently, this research work also presents a study assessing the effect of five locally sourced agricultural based materials (Oat pellet, canola pellet, barley pellet, whole barley grain, whole wheat grain) as sole diet on growth and survival, and their suitability for maintaining a reproducing population of GSL *Artemia franciscana* at low (20 g L^{-1}) salinity. Two separate tests were conducted which involved firstly a nine days small scale (using 500 mL glass bottles) feeding experiment in order to screen and select suitable feed material(s), followed by up-scaling and a mass culture of *Artemia* from the nauplii stage and through the reproductive stage (43 days), using the selected feed material(s). Both the screening and the up-scaled biomass tests showed that feed type influences the growth and survival of the animals, whereby feeding a stock suspension prepared from barley pellet resulted in better performance than feeding with the other feed materials. The up-scaled biomass test confirmed that the barley pellet diet could be used for the culture of actively reproducing biomass of the GSL strain of *Artemia franciscana*. During the reproductive period of the biomass daily nauplii harvest was achieved over a period of 22 days, after which the culture was terminated when nauplii production reduced consistently. Despite low levels of nutrients of the different feed materials, the adults fed with the test feed and the nauplii produced ovoviviparously were found to contain appreciable levels of protein, essential and non-essential amino acids as well as saturated, mono-unsaturated and poly-unsaturated fatty acids in their tissues. In order to achieve higher nauplii production, however, feed manipulation, contrary to feeding with sole diets may yield better results.

The suitability of the ovoviviparously produced nauplii was tested by feeding them directly to catfish *Clarias gariepinus* larvae in comparison with oviparously produced nauplii and decapsulated cysts. Higher survival, better protein efficiency ratio and food conversion ratio were observed in catfish larvae fed with the ovoviviparous nauplii. We conclude that the ovoviviparous nauplii could serve as an alternative live food for larval fish.

The economic viability of using the ovoviviparously produced nauplii at the current production capacity of the developed pilot system versus the use of the imported *Artemia* cysts was analyzed. Cost analysis of producing an individual *C. gariepinus* larva fed with the ovoviviparously produced *Artemia* nauplii was found to be 2.15 USD while the cost involved to produce the fish larva fed with locally shopped imported decapsulated *Artemia* cysts was approximately 0.002 USD. The result of

the analysis clearly shows that at the current capacity of the developed nauplii production system, the cost of feeding anindividual *C. gariepinus* larva is far higher than when the imported decapsulated cysts are utilized. However, there is still a lot of room for optimisation of many aspects of our production system, so that the result of the above comparison may be altered in favour of the use of ovoviviparous nauplii. Moreover, the overall benefits of using the ovoviviparous nauplii should not only be anchored on monetary terms but also on the associated benefits such as fish larval growth, survival rate and postlarval quality, constant and predictable quality of the live food offered to the fish larvae, self-reliance of live food production etc.

Finally, the overall results of this work are discussed in the framework of its objectives. Some limitations and their implications, which may have interfered with validity of the results, are highlighted. In order to enhance production of the nauplii, it is recommended for the system to be optimized and more studies to be conducted on various aspects such as stocking density of the culture, the extent to which the feed types offered affect the performance of the maternal population in terms of fecundity and type of reproduction, and the nutritional quality of the ovoviviparous nauplii.

OPSOMMING

Die soutwater *Artemia* is 'n klein skaaldier wat as lewende voer gebruik word in vis- en skulpviskultuur. Van al die lewende voertipes wat algemeen in akwakultuur gebruik word, is *Artemia* wyd bekend en word hoofsaaklik aanvaar as gevolg van die gemak waarmee dit in broeierye gebruik kan word, hetsy in die vorm van nauplii, vanaf siste uitgebroei of as gedekapsuleerde siste wat verkoop word as van-die-rak produkte. Die verskaffing van siste is bemoeilik weens die diskontinuïteit van *Artemia*-verspreiding in natuurlike biotope, weerkundige skommelinge, klimatologiese veranderinge en moontlike oor-oesing van hulpbronne. Dit het tot onstabiliteit gelei met gevolglike fluktuasies en soms afname in die globale produksie. Daarvolgens is streeksgebonde sistvoorraad op baie plekke verseker deur kunsmatige innokulasie en mensgedrewe produksie in soutdamme. Broeierye in sub-Sahara Afrika het tot dusver staatgemaak op die invoer van produkte van wisselende gehalte, wat die doeltreffendheid van visproduksie nadelig beïnvloed en die mededingendheid van akwakultuur in hierdie streek verminder.

Hierdie navorsingswerk dra by tot die kennis oor alternatiewe maniere vir die voorsiening van *Artemia* as lewende voer vir gebruik in broeierye. Eerstens is literatuur hersien, wat fokus op die algemene oorsig van die status van akwakultuur wêreldwyd en binne die sub-Sahara-streek van Afrika; die taksonomie, biologie, kultuur en aanwendings van *Artemia*; op die taksonomie, biologie en akwakultuurproduksie van die Afrika-katvis *Clarias gariepinus*, en die vereiste van lewendige voer vir hul larwes. Onder andere uitdagings wat akwakultuur in Afrika in die gesig staar, is daar 'n algemene herhalende faktor wat geïdentifiseer word, nl. die onvoldoende of nie-beskikbaarheid van vissaad wat op sy beurt gedeeltelik verband hou met die skaarsheid van lewende voere wat 'n noodsaaklike komponent in die broeikasproduksie van larwes van baie gekweekte visspesies behels.

Tweedens word data aangebied oor 'n studie van lae saliniteit (0 - 32 g L^{-1}) kultuur van *Artemia* met vier stamme van twee biseksuele en twee partenogenetiese populasies. Laboratoriumtoetse is uitgevoer om eerstens die oorlewing van instar I en II by lae saliniteit gedurende 'n 48 uur periode axenies te meet. Die gebruik van die twee instare was gebaseer op die hipotese dat die omringende saliniteit nie die embrio en instar I nauplii van *Artemia* beïnvloed nie, en om te bepaal of die gebruik van instar II nauplii om kultuur te innokuleer oorlewing sal beïnvloed. Soortgelyk is die oorlewing van die *Artemia* by twee innokulasies tye (bv. nauplii en pre-volwasse) xenies oor 'n nege dae periode by hierdie lae saliniteite getoets. Die twee innokulasie tye is gebruik op grond van die hipotese dat mortaliteit gewoonlik verminder as *Artemia* volwassenheid bereik. Resultate van albei toetse het getoon dat die lae saliniteit invloed op oorlewing het ten opsigte van die stamtipes. Met betrekking tot die twee instar ontwikkelingsfases is geen verskil waargeneem as gevolg van die lae saliniteite wat getoets is nie. Met betrekking tot die innokulasie tye is daar ook

geen verskille waargeneem onder die getoetste saliniteite nie, behalwe by laer (5 g L^{-1}) saliniteit waar die laagste oorlewing aangeteken is. Verder het 'n toets oor die effek van lae saliniteit op die voortplantings- en lewenseienskappe getoon dat beide saliniteit en stam invloed op die gemete parameters het. Oor die algemeen is hoë oorlewings waargeneem by daardie lae saliniteite waar oorlewing plaasgevind het, maar die hoogste is by 20 en 32 g L^{-1} (kontrole) saliniteit aangeteken. Algeheel is die beste prestasie in terme van die gemete reproduktiewe en lewenseienskappe waargeneem met die Great Salt Lake (GSL) stam. In die besonder het die GSL-stam die hoogste totale nageslag sowel as die aantal nageslag wat as nauplii geproduseer is. Ons kom tot die gevolgtrekking dat 20 g L^{-1} saliniteit en die GSL-stam die mees geskikte is vir gebruik in massakultuur.

Vervolgens bied hierdie navorsingswerk ook 'n studie aan oor die effek van vyf plaaslik-gebaseerde landbou-gebaseerde materiale as enigste dieet oor groei en oorlewing, en hul geskiktheid om 'n reproduksiebevolking van GSL *Artemia franciscana* by lae (20 g L^{-1}) soutgehalte te handhaaf. Twee afsonderlike toetse is uitgevoer wat eerstens 'n nege dae kleinskaalse (met 500 ml glasbottels) voer eksperiment gebruik het om geskikte voedingsmateriaal (s) te ondersoek en te selekteer, gevolg deur opskaling en massakultuur van *Artemia* vanuit die nauplii-stadium en deur die voortplantingsfase (43 dae), deur gebruik te maak van die geselekteerde voedingsmateriaal (e). Beide die ondersoek en die opgradering van biomassa-toetse het getoon dat die voedingstowwe die groei en oorlewing van die diere beïnvloed, en asook die voeding van 'n voorraadsuspensie wat uit garspille voorberei is, beter prestasie lewer as om met die ander voedingsmiddels te voer. Die opgeskaalde biomassetoets het bevestig dat die garspille-dieet gebruik kan word vir die kultuur van aktief produserende biomassa van die GSL-stam van *Artemia franciscana*. Gedurende die voortplantingstydperk van die biomassa is daaglikse nauplii geoes oor 'n periode van 22 dae, waarna die kultuur beëindig is toe nauplii produksie konsekwent verminder het. Ten spyte van die lae vlakke van voedingwaarde van die verskillende stowwe, het die volwassenes wat gevoed is met die toetsvoer en die nauplii wat ovoviviparies geproduseer is, aansienlike vlakke van proteïene, essensiële en nie-essensiële aminosure bevat, asook versadigde, mono-onversadigde en poli- onversadigde vetsure in hul weefsels. Om egter hoër naupliiproduksie te behaal, kan voedingsmanipulasie, in teenstelling met die voer van slegs enkele diëte, beter resultate lewer.

Die geskiktheid van die ovoviviparіese geproduseerde nauplii is getoets deur hulle direk aan katvis *Clarias gariepinus* larwes te voer in vergelyking met oviparіese geproduseerde nauplii en gedekapsuleerde siste. Hoër oorlewing, beter proteïendoeltreffendheidsverhouding en voeromskakelingsverhouding is waargeneem in katvis larwes gevoer met die ovoviviparіese nauplii. Ons kom tot die gevolgtrekking dat die ovoviviparіese nauplii as alternatiewe lewendige voer vir larwale vis kan dien.

Die ekonomiese lewensvatbaarheid van die gebruik van die ovovivipariese geproduseerde nauplii teen die huidige produksiekapasiteit van die ontwikkelde proefstelsel teenoor die gebruik van die ingevoerde *Artemia*-siste is ondersoek. Die koste analise om ‘n individuele *C. gariepinus* larwe gevoer met die ovovivipariese geproduseerde *Artemia nauplii* te produseer, beloop 2,15 USD, terwyl die koste betrokke om die vislarwe te produseer gevoer met plaaslik ingevoerde dekapsuleerde *Artemia* siste was ongeveer 0.002 USD. Die resultaat van die analise toon duidelik dat die koste van die voer van 'n individuele *C. gariepinus* larwe by die huidige kapasiteit van die ontwikkelde nauplii-produksiestelsel veel hoër is as wanneer die ingevoerde dekapsuleerde siste gebruik word. Daar is egter nog baie ruimte vir die optimalisering van baie aspekte van die produksiestelsel, sodat die resultaat van bogenoemde vergelyking verander kan word ten gunste van die gebruik van ovovivipariese nauplii. Daarbenewens moet die algehele voordele van die gebruik van die ovovivipariese nauplii nie net op monetêre terme geanker word nie, maar ook op die gepaardgaande voordele soos die groei van vis larwes, oorlewingsyfer en post-larwale kwaliteit, konstante en voorspelbare gehalte van die lewende voer wat aan die vislarwes aangebied word, selfstandigheid van lewende voerproduksie, ens.

Ten slotte word die algehele resultate van hierdie werk bespreek in die raamwerk van die doelwitte. Sommige beperkings en hul implikasies, wat moontlik die geldigheid van die uitslae belemmer het, word uitgelig. Om die produksie van die nauplii te verbeter, word dit aanbeveel dat die stelsel geoptimaliseer word en meer studies gedoen word oor verskeie aspekte soos die behuisingsdigtheid van die kultuur, die mate waarin die voertipes wat aangebied word, die prestasie van die moederbevolking beïnvloed in terme van fertiliteit en tipe voortplanting, en die voedingswaarde van die ovovivipariese nauplii.

SAMENVATTING

Het pekelkreeftje *Artemia* is een kleine garnaalachtige, gebruikt als levend voedsel bij de kweek van vis en schaaldieren. Binnen de groep van types levend voedsel die frequent gebruikt worden in de aquacultuur, is *Artemia* algemeen bekend en geaccepteerd, vooral dankzij zijn gebruiksgemak in broedhuizen, hetzij onder de vorm van nauplii die ontloken zijn uit cysten, hetzij als gedecapsuleerde cysten die verkocht worden als kant-en-klare producten. De beschikbaarheid van de cysten wordt bemoeilijkt door de discontinuïteit van de verspreiding van *Artemia* in natuurlijke biotopen, door meteorologische fluctuaties, door klimaatsverandering en door mogelijke overbevissing van de natuurlijke voorraden. Dit heeft geleid tot instabiliteit en tot fluctuaties in, en soms daling van, de globale productie. Bijgevolg werd de regionale bevoorrading van cysten in vele plaatsen verzekerd door kunstmatige inoculatie en door de mens gecontroleerde productie in zoutvijvers. Broedhuizen in sub-Sahara Afrika hebben tot nu toe meestal gebruik gemaakt van import van product van wisselvallige kwaliteit, hetgeen de efficiëntie van de visproductie negatief beïnvloed heeft en de competitiviteit van aquacultuur in deze regio heeft aangetast.

Dit onderzoek draagt bij tot de kennis over alternatieve manieren om de bevoorrading van *Artemia* als levend voedsel in broedhuizen te verzekeren. In eerste instantie wordt een overzicht gegeven van de literatuur, met focus op de algemene status van aquacultuur in de wereld en in sub-Sahara Afrika: de taxonomie, biologie, kweek en toepassingen van *Artemia*; verder ook de taxonomie, biologie en aquacultuurproductie van de Afrikaanse katvis *Clarias gariepinus*, en de noodzaak aan levend voedsel voor diens larven. Naast andere uitdagingen voor de aquacultuur in Afrika, is een vaak terugkerende factor het gedeeltelijk of volledig gebrek aan vislarven voor de uitgroei, wat op zijn beurt verband houdt met het gebrek aan levend voedsel, wat een essentiële component is bij de productie in het broedhuis van de larven van talrijke gekweekte vissoorten.

Vervolgens worden data voorgesteld over een studie inzake kweek van *Artemia* bij laag zoutgehalte (0 – 32 g L^{-1}), waarbij gebruik gemaakt werd van twee bisexuele [Great Salt Lake (GSL) en Vinh Chau (VC)] en twee parthenogenetische [Tuz (TUZ) en Balikun (BLK)] populaties. Eerst werden tests uitgevoerd in het laboratorium, om in axenische omstandigheden de overleving van instar I en II te bepalen bij laag zoutgehalte over een periode van 48 u. Het gebruik van beide instars was gebaseerd op de hypothese dat de omgevingssaliniteit geen invloed heeft op het embryo en de instar I-nauplii van *Artemia*, en om te bepalen of het gebruik van instar II-nauplii om de kweek te inoculeren een effect zou hebben op de overleving. Op gelijkaardige manier werd overleving van *Artemia* van verschillende leeftijd op het tijdstip van inoculatie (nl. nauplii en pre-adulten) getest in xenische omstandigheden over een periode van 9 dagen bij deze lage zoutgehalten. Deze twee leeftijden waren gebaseerd op de hypothese dat de sterfte van *Artemia* gewoonlijk afneemt naar de maturiteit toe. De resultaten van beide tests toonden aan dat het lage zoutgehalte een invloed had op de overleving voor wat de rassen betreft. Wat betreft de twee

ontwikkelingsstadia van instar, werd geen verschil waargenomen als gevolg van de uitgeteste zoutgehalten. Wat betreft de leeftijd van inoculatie, werd evenmin een verschil waargenomen tussen de uitgeteste saliniteiten, behalve bij lagere saliniteit (5 g L^{-1}) waar de laagste overleving werd opgetekend. Verder toonde een test die peilde naar het effect van lage saliniteit op de voortplantings- en levensloopkenmerken aan, dat zoutgehalte en ras een invloed hadden op de gemeten parameters. Over het algemeen werd hoge overleving waargenomen bij die lage saliniteiten waar er overleving was, maar de hoogste overleving werd opgetekend bij een saliniteit van 20 en 32 g L^{-1} (de controle). Over het algemeen werden de meest productieve waarden voor voortplanting en levensloop waargenomen met het ras van Great Salt Lake (GSL). Meer specifiek vertoonde het GSL-ras het hoogste aantal nakomelingen, evenals het hoogste aantal nakomelingen geproduceerd als nauplii. We besluiten dat een zoutgehalte van 20 g L^{-1} en het GSL-ras het meest geschikt zijn voor een massakweek.

Vervolgens omvat dit onderzoek ook een studie die vijf lokaal beschikbare landbouwproducten (haverpellets, canolapellets, gerstpellets, integraal gerstgraan, integraal tarwegraan) evalueert op hun geschiktheid om optimale groei en overleving te garanderen als monodieet voor een zich voortplantende populatie van GSL *Artemia franciscana* bij lage saliniteit (20 g L^{-1}). Twee aparte tests werden uitgevoerd: eerst een kleinschalige voedertest (in glazen flessen van 500 mL) gedurende 9 dagen om geschikte voedermaterialen te screenen en selecteren. Deze test werd gevolgd door een massakweek op grotere schaal die startte met het nauplius-stadium, en doorliep doorheen het reproductiestadium (43 dagen), waarbij gebruik gemaakt werd van de geselecteerde voedermaterialen. Zowel de screening-test als de meer grootschalige biomassa-test toonden aan dat het voedertype een invloed heeft op groei en overleving van de dieren, waarbij het voederen van een stocksuspensie op basis van gerstpellets leidde tot betere resultaten dan het voederen met andere materialen. De grootschalige biomassa-test bevestigde dat het dieet op basis van gerstpellets gebruikt kan worden voor de kweek van een zich actief voortplantende populatie van het GSL-ras van *Artemia franciscana*. Gedurende de voortplantingsperiode van de biomassa werden over een duur van 22 dagen dagelijks nauplii geoogst, waarna de kweek werd stopgezet omdat de nauplii-productie stelselmatig afnam. Ondanks de lage gehaltes aan nutriënten in de verschillende voedermaterialen bevatten de adulte dieren, gevoederd met de testvoeders, en de ovovivipare nauplii behoorlijke hoeveelheden eiwitten, essentiële en niet-essentiële aminozuren, evenals verzadigde, mono-onverzadigde en poly-onverzadigde vetzuren in hun weefsels. Echter, om hogere productie van nauplii te verkrijgen, kan verdere manipulatie van het dieet, in tegenstelling tot het voederen met mono-diëten, betere resultaten opleveren.

De geschiktheid van de ovovivipaar geproduceerde nauplii werd getest door ze rechtstreeks te voederen aan larven van katvis *Clarias gariepinus*, in vergelijking met ovipaar geproduceerde nauplii en gedecapsuleerde cysten. Hogere overleving, betere eiwit-efficiëntie-ratio en voederconversie-ratio werden bekomen met katvislarven gevoederd met ovovivipare nauplii. We

besluiten dat de ovovivipare nauplii een geschikt alternatief levend voedsel voor larvale vis kunnen zijn.

We analyseerden tenslotte de economische haalbaarheid van het gebruik van de ovovivipaar geproduceerde nauplii bij de huidige productiecapaciteit van het ontwikkelde pilootsysteem, versus het gebruik van geimporteerde *Artemia*-cysten. Een kostanalyse berekende de kost van de productie van een individuele larve van *C. gariepinus*, gevoederd met ovovivipaar geproduceerde *Artemia*-nauplii, op 2,15 USD, terwijl de productiekost bij het gebruik van ingevoerde gedecapsuleerde *Artemia*-cysten bij benadering 0,002 USD is. Deze berekening toont duidelijk aan dat bij de huidige productiecapaciteit van het ontwikkelde productiesysteem, de productiekost van een individuele larve van *C. gariepinus* veel hoger ligt dan bij gebruik van ingevoerde gedecapsuleerde cysten. Er is echter nog veel ruimte voor verbetering van talrijke aspecten van ons productiesysteem, zodat de resultaten van bovenstaande vergelijking kunnen wijzigen in het voordeel van het gebruik van ovovivipare nauplii. Bovendien zijn de voordelen van hun gebruik niet alleen gelinkt aan monetaire aspecten, maar ook aan ermee verbonden voordelen inzake de larvale groei van de vis, overleving en postlarvale kwaliteit, constante en voorspelbare kwaliteit van het levend voedsel aangeboden aan de vislarven, zelfredzaamheid inzake productie van levend voedsel, enz.

Tot slot worden de algemene resultaten van dit werk besproken binnen het kader van de gestelde objectieven. Sommige beperkingen en hun implicaties, die geïnterfereerd kunnen hebben met de validiteit van de resultaten, worden belicht. Om de productie van de nauplii te bevorderen, is het aanbevolen dat het systeem wordt geoptimaliseerd en dat meer studies worden uitgevoerd inzake verschillende aspecten zoals de stockeringsdensiteit van de kweek, de mate waarin de toegediende voedertypes de prestaties van de moederpopulatie beinvloeden op het vlak van fecunditeit en type van reproductie, en de nutritionele kwaliteit van de ovovivipare nauplii.

This dissertation is dedicated to God Almighty

ACKNOWLEDGEMENTS

This dissertation would not have been possible without the guidance and inputs of certain persons and institutions that directly or indirectly played a role in the overall success. I would therefore like to express my sincere gratitude to:

God Almighty through Jesus Christ for the grace given me to accomplish the task which looked insurmountable. Indeed, with you, all things are possible!

My supervisors, Dr Khalid Salie and Prof dr Gilbert Van Stappen for their continuous guidance and support throughout my doctoral work. Many thanks for your kindness, patience and always making time from your tight schedule to make invaluable input and advices in making my PhD journey a success and such an incredible experience.

My reading and examination committee. I sincerely appreciate all your efforts, comments and recommendations. I am highly indebted.

The Aquaculture Department of Stellenbosch University, South Africa and the Laboratory of Aquaculture & *Artemia* Reference Center, Ghent University, Belgium for the opportunity given to me and all the assistance rendered through your hard working and supportive staff. I consider it a privilege to be a part of these great institutions of learning. I especially like to thank Em Prof dr Patrick Sorgeloos, Prof dr ir Peter Bossier, Proff Danie Brink, Kennedy Dzama, Ms. Gail Jordaan, Mr Henk Stander, Mr Anvor Adams, Mr Christ Mahieu and Ms Anita De Haese for the tremendous support you gave to me throughout the period of this study.

The Executive Director, management and entire staff of the National Institute for Freshwater Fisheries Research, New Bussa, Nigeria and the West African Agricultural Productivity programme (WAAPP – Nigeria) for the opportunity and all the support given to me to pursue this goal.

My family Elvis, Pwadeido (Mine), Nathan (Pedeino) and Talmon (Arhyel) for your support, patience and understanding. Although we have always been together through the entire journey, yet it felt like we were far apart. It only dawned on me that within the period, my children “grew in wisdom and statue” and I didn’t even realize it. You all are special and particularly my wife (Elvis) who always pray for me and taught the children to do same!

My parents Mr. Lema Sambo Mshelbwala and Late Mrs Deborah Lema who taught me all of the virtue which I have acquired in life and to keep focus in all things. May God bless your souls.

Aaron, Saratu, Nao’mi, Watirahyel, David, and Jummai for the privilege to be a part of you in this journey of life and for all your good wishes. You are a bunch of wonderful siblings. God bless you and your families.

All my colleagues at work, particularly Dr SI Ovie, who put the passion in me to pursue my dreams especially in this area of study. Also, Dr Godfrey Nwabeze and Dr Lyneth Ibiyo for your contribution during the course of writing this thesis.

All the beautiful and kind-hearted people at Stellenbosch Baptist church, South Africa. I particularly want to appreciate pastor Byron de Klerk, “Ouma” Lynneth Miln, Eugine and Irma Van Royen, Ellaine De Goede and Inge Wissels. You all are indeed a family and I bless the day our paths

crossed. Thank you for allowing God to use you in very special ways to touch lives. Keep doing the good work for your rewards await you in heaven.

The Lehmans (Papa Ulli and Mama Heide). I always wonder how you manage to make life so easy for so many strangers in a foreign land. You have positively impacted the lives of many, especially my family. Through your ministry (International students' ministry), I was priviledged to meet with many amazing individuals whom have also been a great source of blessing to me. God bless each one of you in return!

The ECWA students' fellowship of Stellenbosch University (ESFUS) and my home church (ECWA GoodNews, New Bussa, Nigeria), for all your prayers and good wishes.

All my friends home and abroad for being a part of this incredible journey. For some, we've lost touch for quite so long due to the demands to accomplish this task. But I am optimistic that we'll soon link up again. THANK YOU FOR YOUR UNDERSTANDING!

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND UNITS

<	Less than
>	Greater than
≤	Less than or equals to
≥	Greater than or equals to
˚C	Degree celcius
%	Percent
m^2	Meter square
m^3	Meter cube
µg	Microgram
µL	Microlitre
µm	Micrometre
±	Approximately
$^{-1}$	Per
min	Minute
s	Second
h	Hour
ΔpH_i	Intra-cellular pH switch
AAS	Ascorbic acid 2-sulfate
AD	Anno Domini
ARC	Laboratory of Aquaculture & *Artemia* Reference Center
ANOVA	Analysis of variance
AOAC	Association of analytical chemists
AQC	6 – amino quinolyl-N-hydroxy succimidyl carbamate
BLK	Balikun strain of parthenogenic *Artemia*
BP	Barley pellet
BPE	Before present era
BBP	Biomass fed barley pellet
BOP	Biomass fed oat pellet
BWBG	Biomass fed whole barley grain
CFU	Colony forming unit
C. gariepinus	*Clarias gariepinus*
cm	Centimetre
CP	Canola pellet
CTU	Can Tho University, Vietnam
DEC	Decapsulated cysts
DHA	Docosahexaenoic acid

DOCA	Deoxycorticosterone acetate
EFA	Essential fatty acid(s)
EPA	Eicosapentaenoic acid
FAA	Free amino acid(s)
FASW	Filtered and autoclaved seawater
FAME	Fatty acid methyl ester(s)
FAO	Food and Agriculture Organization of the United Nations
FCR	Food conversion ratio
FDF	Federal Department of Fisheries
FT	Falcon tube(s)
GART	Gnotobiotic *Artemia* culture system
GDP	Gross domestic product
GP_4G	Trehalose, glycerol and guanosine (5') tetraphospho (5') guanosine
GSL	Great Salt Lake strain of *Artemia franciscana*
g	Gram
gL^{-1}	Gram per litre
ha	Hectare
HCG	Human chorionic gonadotropin
HUFA	Highly unsaturated fatty acid(s)
K	Condition factor
KES	Kenyan shilling
kg	Kilogram
km	Kilometre
LVS 3	*Aeromonas harveyi* strain 3
L	Litre
m	Metre
mg	Milligram
mL	Millilitre
mm	Millimetre
Na, K-ATPase	Sodium potassium-Adenosine triphosphatase
NGN	Nigerian Naira
OD	Optical density
ODC	Ornithine decarboxylase
OP	Oat pellet
OVV	Ovoviviparous nauplii
OVP	Oviparous nauplii

PDA	Photodiode array
PER	Protein efficiency ratio
P.R. China	People's Republic of China
P statistical	p-value
p26, Hsp70 and 90	Stress proteins
rpm	Rotations per minute
SFB	San Francisco Bay
SGR	Specific growth rate
Stdev	Standard deviation
TC	Total cost
TFC	Total fixed cost
TL	Total length
TVC	Total variable cost
UPLC	Ultra performance liquid chromatography
USD	United States Dollar
VC	Vinh Chau strain of *Artemia franciscana*
VF	Vinh Chau feed
W	Watt
WAAPP	West African Agricultural Productivity Programme
WBG	Whole barley grain
WHR	Water replacement hypothesis
WRS	Water recirculation system
WWG	Whole wheat grain

LIST OF TABLES

LIST OF FIGURES

Chapter 1

Introduction and thesis outline

CHAPTER 1

INTRODUCTION AND THESIS OUTLINE

1.1. Fish larvae and their requirement for live food

In fish husbandry, it is generally known that the larvae of several marine and freshwater species require live food at first feeding. Several studies with the larvae of different species have revealed that live food is indeed an essential component for optimal growth and survival. This has been attributed mainly to factors such as an undeveloped digestive system, poor vision and undeveloped chemo- and mechano-receptors (Mukai, Tuzan, Lim, Wahid, Sitti Raehanah & Senoo, 2008; Hecht, 2013a; Paray, Haniffa, Innocent & Rather, 2016). The African catfish (*Clarias gariepinus* Burchell, 1822), a commercially important species in several regions in sub-Saharan Africa, and hence the fish of interest in this thesis, essentially requires live food at the onset of exogenous feeding. Studies to assess the earliest possible weaning time and the performance for this species reported that in order to attain high survival rate and optimal growth, their larvae essentially require live food for a certain period before they are fed with crumbles of commercial diet (Verreth & Van Tongerine, 1989; Onura, Broeck, Nuvejan, Muendo, & Van Stappen, 2018).

Among the live food types which are commonly used in aquaculture, the brine shrimp *Artemia* is widely known and accepted. This is mainly due to the ease with which it can be used either in the form of nauplii freshly hatched from cysts, or as decapsulated cysts which are sold as off-the-shelf products (Lavens & Sorgeloos, 2000). Additionally, several larval studies have reported obtaining higher survival and better growth responses when feeding *Artemia* compared to other live food types and formulated diets. Since the discovery (in the 1930s) of *Artemia* as ideal food for larvae of many aquaculture organisms, there has been increased demand for their cysts globally (Van Stappen, 1996a). However, reports have also shown that *Artemia* habitats which are the main supply sources of cysts product, have been vulnerable to meteorological fluctuations and climatological changes (Lavens & Sorgeloos, 2000). This, coupled with possible overharvesting of resources, has led to instability and resulted in fluctuations and sometimes reduction of the global production (Figure 1.1). Consequently, prices in markets globally have continued to soar (Maldonado-Montiel, Rodriguez-Canche & Olvera-Novoa, 2003). It has been reported that a kilogram of good quality *Artemia* cysts can cost up to 200–250 USD in the world market (Ndavano, 2012).

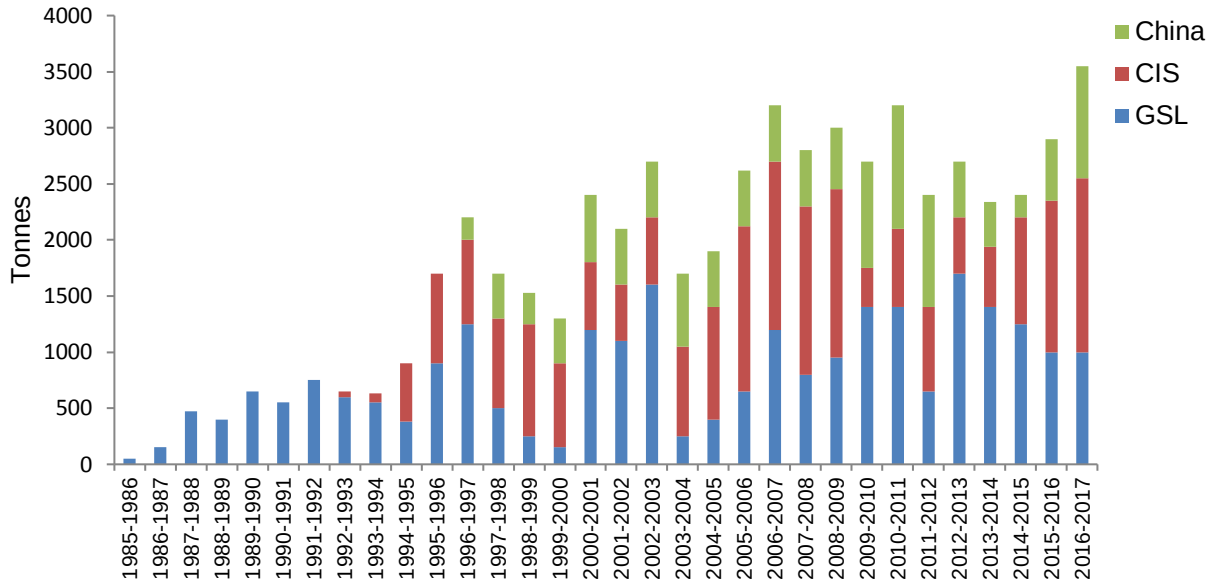

Figure 1.1: Global *Artemia* production from 1985 - 2017. Source: Hasan 2016. GSL = Great Salt Lake, CIS = Central Asia

1.2. Problem statement

Due to some limiting factors such as means of dispersal and absence of anatomical defence mechanisms in *Artemia*, their distribution worldwide is discontinuous (Van Stappen, 1996b). Regional cyst supplies, particularly where *Artemia* is not found existing in nature, have thus often been ensured through artificial inoculation and man-managed production of *Artemia* in saline water bodies such as solar saltworks, to avoid or limit importation of expensive cysts product. In sub-Saharan Africa, natural *Artemia* resources are poorly documented (Kaiser, Gordon and Paulette, 2006) and managed production in solar saltworks is hardly practiced (Ogello, Kembenya, Githukiya, Nyonje & Munguti, 2014). As a result, many hatcheries in sub-Saharan Africa have so far relied on importation. The need for importation of this product in order to meet local demands, coupled with incidences of low quality cysts product (Lavens and Sorgeloos, 2000) and limited knowledge on appropriate use of *Artemia* cysts, has adversely affected fish production efficiency and reduced the competitiveness of aquaculture in this region. Hence the need to intensify efforts for finding alternative means for the supply of *Artemia* within the region.

1.3. Research objectives and thesis outline

In *Artemia*, two modes of reproduction (oviparity and ovoviviparity) are recognized. Contrary to the oviparous mode of reproduction which ensures cysts production, the alternative ovoviviparous mode whereby the parent animals release live, free swimming larvae, offers the opportunity for the production of nauplii with a possibility for continuous harvest. It has been highlighted that the technique for the controlled production of ovoviviparous nauplii offers the prospect for application in aquaculture hatcheries, while ensuring independence from the international cysts market as well

as providing a far better control of the quality of this live food product (Lavens & Sorgeloos, 1987). The overall aim of this thesis was therefore to develop a cost-effective indoor pilot system for the production and continuous or semi-continuous harvest of nauplii via the ovoviviparous mode over an extended period of time. At present, studies which reported nauplii production via the ovoviviparous mode of reproduction in *Artemia*, and its value for aquaculture, are scarce. Not only will this thesis be the first study to provide information on the potential of such a system for continuous harvest of the ovoviviparous nauplii, but also to assess the direct feeding of these nauplii to African catfish (*C. gariepinus*) larvae. This was achieved by identifying specific objectives.

1) Firstly, the best salinity level was selected (being one of the most important biotic elements in *Artemia* culture which influences reproduction) and a suitable strain which supports ovoviviparity.
2) Secondly, a low cost agricultural feed material was selected to serve as alternative to phytoplankton or to commercially produced *Artemia* feed, which would support long-term survival, growth and reproduction of the population.
3) Finally, the nutritional value of ovoviviparously produced nauplii was assessed by studying the performance of African catfish larvae (*C. gariepinus*) fed these nauplii, in comparison with commercially available decapsulated cysts and freshly hatched (oviparous) nauplii.

The above described work was subdivided in the following chapters:

Chapter 2: Literature review. This chapter aimed at giving a general overview on the status of aquaculture globally and within the sub-Saharan region of Africa, on the taxonomy, biology, culture and applications of *Artemia*, and on the taxonomy, biology and aquaculture production of *C. gariepinus*, and the requirement of live food for their larvae.

Chapter 3: Survival, reproductive and life trait responses of four *Artemia* strains in low salinity. This chapter aimed at testing the influences of low salinity on survival, reproductive and life trait parameters of four *Artemia* strains in order to identify the most suitable (low) salinity as well as the most suitable *Artemia* strain for application in mass culture, ensuring high survival and ovoviviparity. The choice of low salinity as culture medium was inspired firstly by the reports that low salinity could induce nauplii production while supporting good physiological conditions in *Artemia* (Lavens & Sorgeloos, 1991; Agh, Abatzopoulos, Van Stappen, Razawi Rouhani & Sorgeloos, 2007; Agh, Van Stappen, Bossier, Seperhi, Lofti, Razawi Rouhani & Sorgeloos, 2008). Secondly, hatcheries in sub-Saharan Africa located far from the coast, often use different commercial salts or dissolve crude sea salt to formulate artificial seawater for hatching *Artemia* cysts; by lowering the salinity level, the quantity and consequently the cost of salts required for the

formulation could also be reduced. Laboratory tests were performed, firstly, to measure axenically the survival of instar I and II at low salinity over a period of 48 h using 50 mL glass screw cap bottles.The use of the two instars was based on the report that ambient salinity does not affect the emerging embryo and instar I nauplii of *Artemia* (Lee and Watts, 1994), and to investigate the hypothesis that using instar II nauplii to inoculate a culture at low salinity would not have an effect on survival. Similarly, survival of the *Artemia* at two inoculation ages (i.e. nauplii and pre-adult) was tested xenically over a period of nine days at the low salinities using 500 mL glass bottles. The two inoculation ages were used based on the report that the rate of mortality usually reduces as *Artemia* attains maturity (Vanhaecke, Siddall and Sorgeloos, 1984), and to assess the hypothesis that using the pre-adult *Artemia* to inoculate the culture will result in higher survival. Afterwards, 50 mL falcon tubes were used to assess life traits and reproductive traits of the four *Artemia* strains at low salinity.

Chapter 4: Performance of the Great Salt Lake strain of *Artemia franciscana* fed with low cost agricultural material as sole diet. This chapter aimed at identifying locally available agricultural material which could be used as a suitable low-cost feed for culture of the *Artemia*. It assessed the effect of five locally sourced agricultural based materials as sole diet on growth and survival, as well as their feasibility for rearing of reproductive biomass of the Great Salt Lake (GSL) *Artemia franciscana* at low salinity (20 g L^{-1}). The choice of the *Artemia* strain and salinity used were based on findings of the previous chapter. In the study, two separate tests were conducted. Firstly, a small scale feeding experiment in 500 mL glass bottles was performed over a period of nine days in order to measure survival and growth of the GSL *Artemia.* This was followed by up-scaling and mass culturing of the *Artemia* in a constructed production unit consisting of six culture tanks (37 cm x 26 cm x 25 cm; 21 L) over a period of 43 days using selected feed material(s) from the small scale test. The latter experiment ensured observations which covered the life period from the nauplii through the reproductive stages, whereby survival, growth and production parameters were measured.

Chapter 5: Ovoviviparously produced *Artemia* nauplii are a suitable live food source for the larvae of the African catfish (*Clarias gariepinus* Burchell, 1822). In this chapter, the suitability of ovoviviparously produced nauplii as a direct live food source for *C. gariepinus* larvae was tested in comparison to oviparously produced nauplii and decapsulated cysts. A feeding trial was conducted over a period of 7 days to test the performance of the fish larvae using glass aquaria tanks with a fish holding area within the tanks each measuring 20 cm x 9 cm x 7 cm; and culture water volume of 5 L. The *Artemia* production system which was used for mass culture in chapter 4 was maintained to serve as the main source of the ovoviviparous nauplii. The oviparous nauplii were hatched daily while the decapsulated cysts were purchased from commercial suppliers. All of the three *Artemia* feed types belonged to the GSL strain of *Artemia franciscana.*

Chapter 6: Comparative economic analysis of the use of imported decapsulated *Artemia* cysts versus ovoviviparously produced *Artemia* nauplii for feeding larvae of *Clarias gariepinus*. In this chapter, a cost-benefit analysis was made to compare the use of imported decapsulated cysts of *Artemia* by local hatcheries in the sub-Saharan area with the alternative on-site production and use of ovoviviparous nauplii.

Chapter 7: General discussion and conclusions. This chapter discussed the overall results of this thesis in the framework of the research objectives, and presented recommendations for future research.

References: Contains all citations mentioned in this thesis. The Harvard referencing method was adopted.

Chapter 2

Literature review

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

It is a common knowledge that the total global stocks of many commercially important aquatic species have been depleted. Among others, over-exploitation of the natural aquatic resources has been identified as the major cause for this depletion. Within half a century, a huge impact on the aquatic ecosystems has come from rapid and continuous increase in fishing intensity which led to the depletion of resources, the degradation of the environment and which has influenced the evolution of supply, demand and prices (Garcia & Newton, 1995). The fishing industry has continued to face additional challenges such as the current growing concern about the effects of climate change on aquatic systems. Studies have shown how increases in climate change variables have influenced fish populations, distribution and aquatic ecosystems in general (Cochrane, De Young, Soto & Bahri, 2009; Engelhard, Righton & Pinnegar, 2014). The ever-increasing human population is linked with this increased pressure on resources (Engelhard *et al.,* 2014). Recently, the total human population has been estimated at 7.3 billion with projection to reach 9.7 billion by 2050 (Kamaraj, Kathiravan & Jayakumar, 2014; United Nations, 2015). Rice and Garcia (2011) highlighted that an estimated more than 2 billion people are supplied with at least 20 % of their average *per capita* intake of animal protein from fish; hence the question: "can the caloric and dietary protein needs of future populations be met?". Apparently, such question poses responsibilities among stakeholders to seek solutions to the challenges that the fishing industry is faced with. In order to meet the requirement, an additional production of 75 million tonnes (~50 %) from capture fisheries and aquaculture to the present over 150 million tonnes (FAO, 2017b) has been proposed (Rice & Garcia, 2011). Barange, Merino, Blanchard, Scholtens, Harle, Allison, Allen, Holt and Jennings (2014) conditionally asserted that if *per capita* fish consumption will be maintained coupled with the ongoing technological development in the aquaculture industry, the projected global fish demands by 2050 could be met despite the corresponding increases in human population.

It was in a bid to finding solutions to the challenges of depleting stocks in the natural aquatic ecosystems, and also the efforts in meeting the human requirement for sufficient and sustainable fish protein intake, that the aquaculture industry emerged. Over the years, aquaculture has progressed steadily, bridging the gap with the stagnating capture fisheries and showing promising signs that the future demand for fish food can be met (Figure 2.1).

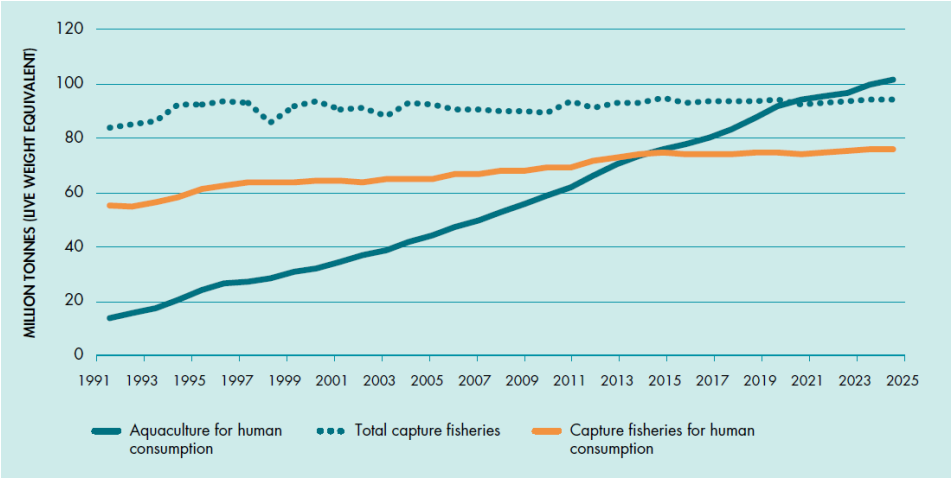

Figure 2.1: Global capture fisheries and aquaculture production to 2025. Source: FAO (2016)

2.2. Aquaculture

2.2.1. The evolution of aquaculture

Aquaculture covers a large variety of activities, which include among others the production of microscopic and macroscopic aquatic plants, ornamental fish, bait fish, a variety of invertebrates, and of recent the polyculture of fish and terrestrial plants commonly referred to as aquaponics. The historic account of the culture of finfish, shrimp and molluscs that are reared as food for human consumption was given in an extensive review by Tidwell (2012). Briefly, he traced it as a relatively long history from the pioneering Chinese and Egyptians who may have developed some form of aquaculture as far back as 3,500 to 4,000 years before present era (BPE) through the various processes associated with spawning and rearing a variety of both marine and freshwater fish to enhance wild capture fisheries, the foundations upon which the science of aquaculture is based.

“Aquaculture continued to expand prior to the mid 1800s with the introduction of different fish species (mainly carp and trout) into various regions from their local source. In the latter half of the nineteenth century, enthusiasm for recreational fishing coupled with increasing numbers of reports that many freshwater and marine stocks were being negatively impacted by overfishing led to the development of private and public hatcheries in Europe and North America. It wasn’t until the mid-twentieth century that the art of aquaculture developed into what exists today as a complex multidisciplinary science that now features over 200 species under culture (including invertebrates and finfish). However, significant progress in the development of the techniques required for spawning and rearing a variety of species had been developed by the end of the nineteenth century” (Tidwell, 2012). Although, the annual growth has declined from 10.8 and 9.5 % in the 1980s and 1990s respectively, to 5.8 % during the period 2001 and 2016 (FAO, 2018), aquaculture is still referred to as the fastest growing food-producing sector in the world (Hebrew University of Jerusalem, 2017). The total global aquaculture production in 2016 was 110.2 million tonnes of which 80 million tonnes (73 %) is food fish (FAO, 2018). The dominant group in terms of production

are the finfish (FAO, 2018), whereby the freshwater fishes contribute 47.3 million tonnes (Hasan, 2016).

2.2.2. Challenges for aquaculture development in Africa

Africa's contribution to aquaculture growth between 1998 and 2007 was a four-fold increase in production from 43,000 to slightly over 183,000 tonnes, with an average yearly growth of 14.45 % (Satia, 2010). In 2016, Africa's total production was 1, 982 tonnes (FAO, 2018). Since the decline in the global annual growth, a small number of individual countries, particularly in Africa still maintained double-digit growth from 2006 to 2010 (FAO, 2018). This has been attributed to the emergence and intensification of small and medium scale enterprises (NEPAD, 2013). In Africa, the bulk of production (93 %) comes from freshwater aquaculture production and is predominantly the culture of indigenous species of Tilapia and African catfish (*Clarias gariepinus*), with catfish contributing 49 % of total production in 2007 (Satia, 2010). A graphical representation of the comparison between the world's and Africa's production in terms of capture fisheries and aquaculture from historical data, both as in the current situation and future trends, is shown below (Figure 2.2).

Despite the progression in aquaculture development in Africa, the continent is yet to reach its full potential compared to other regions on the globe. Several authors have attributed this to the challenges which have been considered as bottlenecks in both the freshwater and marine aquaculture. Among the identified challenges a common re-occurring factor is the insufficiency or non-availability of fish seed (Fakoya, Owodeinde, Jimoh & Akintola, 2005; Megahed & Aly, 2009; Aboua, 2017). This factor in turn has been partly associated to the scarcity of live food which is an essential component in the hatchery production of the larvae of many cultured fish species.

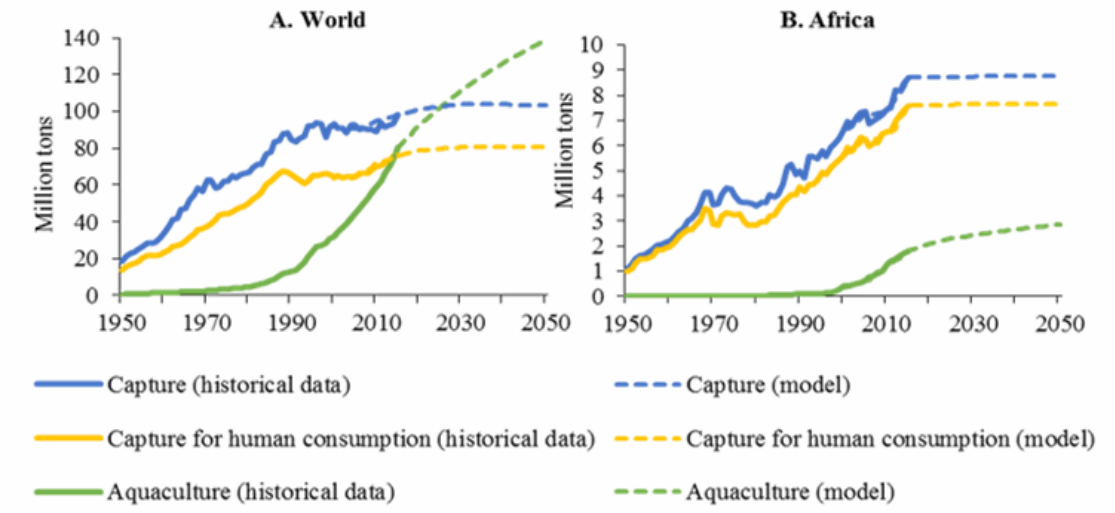

Figure 2.2: World (A) and Africa (B) capture fisheries and aquaculture production, 1950 – 2050.
Source: Chan, Tran, Sulser, Phillips, Batka, Chimatiro, and Dickson (2017)

2.2.3. Fish larvae production and requirement for live food

The role of fish larvae production in the growth of the aquaculture industry over the past four decades cannot be overemphasized. However, hatchery production of larvae using domesticated brood stock is also associated with its challenges; in particular, the high mortality rate of the larvae. High mortality in aquatic larvae of many species has generally been linked to their tiny size and consequently their food requirements (Schoo, Aberle, Malzahn, Schmalenbach & Boersma, 2014). Studies on the survival of aquatic larvae in relation to food availability and density have been documented (Connell & Raymond, 1970; Olson & Olson, 1989; Fiksen & Jørgensen, 2011). When spawning occurs in the wild, the larvae feed on microscopic organisms which are referred to as natural live food (Treece & Davis, 2000). These organisms freely inhabit both the marine and freshwater environments. They include micro algae such as diatoms and flagellates, and zooplankton such as rotifers, cladocerans and copepods (Treece & Davis, 2000). Conversely, larvae produced in the hatchery are restricted only to the type of food offered to them. It is known that freshly hatched larvae of most fish species possess undeveloped physiological systems. At this developmental stage, they are characterized with poor processes of ingestion, absence of a complete digestion enzyme system and weak sensory organs such as the vision (optical receptors), taste buds and olfactory organs (chemo-receptors) and lateral lines (mechano-receptors) (Mukai *et al*., 2008; Hecht, 2013b; Paray *et al*., 2016). Fish larvae at this stage of growth require a food source with certain characteristics. This includes motility with good contrast to attract and trigger predation, be easily digestible (e.g. constituting a large amount of amino acids and oligopeptides rather than indigestible complex protein molecules). Also, such food should be spread in the rearing tank in such a way as to allow easy and frequent contact with the fish larvae due to their weak swimming activity (Lavens & Sorgeloos, 1996; El-Dahhar, Salama, El-Greesy & Shaheen, 2013). In contrast, alternative formulated feeds which are used in many hatcheries have been considered inadequate for rearing of these larval organisms (Rice, Bengtson & Jaworski, 1994; Akbary, Hosseini, Imanpoor, Sudagar & Makhdomi, 2010). Several studies have reported on the consequences of larval feeding with alternative food sources. These studies have shown that when larvae are fed with the formulated diet, the results found are usually unfavourable such as low survival, growth retardation, lower juvenile quality, high size variability and skeletal abnormalities (Le Ruyet, Alexandre, Thebaud & Mugnier, 2007; Akbary *et al*., 2010; Sales, 2011). Using a meta-analysis of published results (47 studies conducted with 27 freshwater fish species) to quantify differences in mortality and growth of freshwater fish larvae when live food was replaced by compound diets at first feeding, Sales (2011) showed that larvae fed on compound diets have a 2.5 times higher chances to die than those fed on live food. A similar negative effect was found on growth.

2.2.4. Limitations of the continuous production of zooplankton organisms

Unlike the commercially produced artificial feed, there is a dearth of information on the production and use of live food for larval stages of many freshwater and brackishwater fish and shell fish (Hasan, 2016). Although zooplankton organisms are commonly found in the wild (Kadhar, Kumar, Ali and John, 2014), they are usually required in large quantity for intensive larval culture (Treece & Davis, 2000). Continuous harvest from the wild for feeding cultured fish larvae is impracticable and may not necessarily guarantee sustainable availability and sufficient supply particularly when needed during breeding in the hatchery. On the other hand, controlled intensive culture of these organisms is similarly limited by their low yield especially in the freshwater species. Stottrup and McEvoy (2002) highlighted that despite some successes recorded in the culture of *Brachionus calyciflorus*, the culture of this freshwater rotifer species does not have the same impact on aquaculture as its marine and brackish water counterparts *Brachionus rotundiformis* and *Brachionus plicatilis*. It is noteworthy that a female amictic *B. plicatilis* has an average life span of only 7.09 days during which it can only produce 17-24 eggs in its entire lifetime (Stottrup & McEvoy, 2002). Treece and Davis (2000) also asserted that the highest reproduction rate of 21 offsprings per female per week was recorded when a pure diet of *Isochrysis galbana* was fed to rotifers. In a study by Ovie (1997), intensive culture of freshwater zooplankton yielded peak densities of 15,462 individuals L^{-1} of *Moina micrura* in a culture volume of 50 m^3 in 12 days; 7,345 individuals L^{-1} of *Diaphanasoma excisum* in a 40 L tank in 4 days and 4,500 individuals L^{-1} of *Brachionus calyciflorus* in 40 L in 3 days. These figures are indicative of low fecundity and they fall short of the huge numbers of rotifers which are required in the billions each day for raising marine fish larvae in commercial hatcheries (Stottrup & McEvoy, 2002). The amounts needed range from 20,000 to 100,000 rotifers per fish larva during the 20–30 days of culture (Stottrup & McEvoy, 2002). The cost of artificially produced rotifer eggs as alternative for daily production of the live organisms has also been asserted to be high which makes it difficult to be adopted in hatcheries (Gopakumar, 2009). As for copepods, aside of requiring large volumes for culture, most adult copepods rarely exceed 100 individuals L^{-1} in density. Generally, the use of zooplankton results in sub-optimal nutrition of the larvae resulting in low survival compared to *Artemia* (Hasan, 2016).

2.3. *Artemia*

2.3.1. *Artemia* as live fish food

Artemia, also referred to as brine shrimp, has been known to man for many centuries even going back to the 10th century AD for Urmia Lake, Iran and the records of Schlösser in 1757 in England (Asem, 2008; Asem & Eimanifar, 2016). *Artemia* is not a natural diet for fish larvae (Lavens & Sorgeloos, 2000); this is attributed to its inhabitation of high salinity environments away from predators. However, it has been reported that certain fish species such as the cyprinodont *Aphanius* sp. that tolerates salinities exceeding 150 g L^{-1} together with other species such as the

mullet (*Mugil* sp.), milkfish (*Chanos* sp.), Tilapia and several crustacean species such as *Palaemonetes,* predate heavily on brine shrimp in saltpans and salt lakes (Van Stappen, 2002). The use of *Artemia* as live food became famous when it was discovered to be an excellent food source for fish larvae (Van Stappen, 1996b). In larviculture, both the cysts (dormant embryo) and freshly hatched nauplii of *Artemia* can be used. Cysts are used in decapsulated form, i.e. after removal of the indigestible chitinous outer cyst shell (chorion) by a simple treatment with a OCl^- solution. The availability and convenience with which the nauplii and cysts are used as food sources for fish and shellfish larvae have made it popular. Considering the parameters for selection of larval food (Lavens and Sorgeloos, 1996), it is clear that *Artemia* is often a more suitable diet for use in larviculture than other live food sources such as phyto- and other zooplankton, and commercially produced artificial feed. From the point of view of the fish culturist (except for cost effectiveness), *Artemia* cysts are convenient for use due to their commercial availability, easy use, and the fact that both cysts (decapsulated) and freshly hatched nauplii can be used to feed fish larvae (Sorgeloos, Dhert & Candreva, 2001). This underscores *Artemia* versatility for application in larviculture. From the point of view of the fed larvae, *Artemia* has also been seen as a good food source due to its motility (in the case of nauplii) within the fish culture medium, high acceptability and good nutritional components which are beneficial to the fish larvae.

2.3.2. Forms of *Artemia* used in larviculture

- **Freshly hatched instar I nauplii**

The nauplii of *Artemia* are usually hatched from the cysts before they are fed to the predator fish larvae. Under optimal hatching conditions (i.e. oxygen level above 2 mg L^{-1}, temperature range of 25 – 28 ˚C, salinity range of 5 – 35 g L^{-1}, pH between 8 – 8.5 and strong illumination of about 2,000 lux at water surface), cysts are incubated for 12 – 20 h after which free swimming nauplii are released (Van Stappen, 1996a; Van, Van Hoa, Bossier, Sorgeloos & Van Stappen, 2014) (Figure 2.3). *Artemia* nauplii are often preferred over other types of live food because they result in better larval growth and reduced mortalities (Hecht & Appelbaum, 1987).

- **Decapsulated cysts**

Direct use of the cysts as feed requires that they are in their decapsulated form. Decapsulation involves a short-term exposure of the raw cyst material to a hypochlorite solution in order to remove the outermost chitinous shell (chorion) that encysts the dormant *Artemia* embryo (Figure 2.3).

- **Umbrella stage**

During hatching of the *Artemia* cysts, after the cyst shell bursts (usually referred to as breaking), the embryo which is still surrounded by the hatching membrane emerges. While still hanging

underneath the empty shell, the emerged embryo completes the differentiation of the pre-nauplii stages before the instar I nauplius is hatched. This stage is referred to as the umbrella stage (Figure 2.3) and more recently, it is commonly used in the hatchery production of mudcrab in Vietnam.

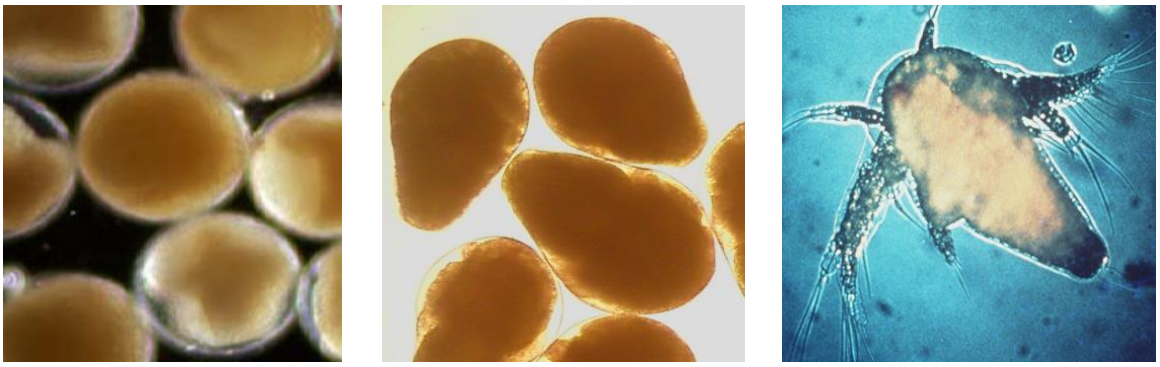

Figure 2.3: Decapsulated cysts (left picture), umbrella stage (middle picture) and instar I nauplius (right picture) of *Artemia*. Source: Sorgeloos (2016)

2.3.3. The biology of *Artemia*

2.3.3.1. Morphology and taxonomy

The genus *Artemia* is a branchiopod crustacean with adult size of ±1 cm in length (Van Stappen, 1996b). It has been described as a primitive arthropod with a segmented body consisting of 19 segments, of which the 11 thoracic segments have paired appendages which perform several functions such as locomotion, gill function, and filtration of food particles (Dobbeleir, Adam, Bossuyt, Bruggeman & Sorgeloos, 1980). The next two segments carry the reproductive organs and the last, abdominal segments lead to the tail (see Van Stappen, 1996b; Criel & Macrae, 2002 for a full description on the morphology and structure of *Artemia*). There are six generally recognized bisexual (zygogenetic) species and numerous parthenogenetic populations having different ploidy levels (Asem, Rastegar-Pouyani & Rios-Escalante, 2010; Dhont & Van Stappen, 2003) (Table 2.1). These species have been defined by the criterion of reproductive isolation (Browne & Bowen, 1991).

Table 2.1: *Artemia* taxonomy, species and their location

Taxonomy	Species and their location
Domain: Eukaryota	*A. salina* Linnaeus, 1758: Mediterranean area
Kingdom: Metazoa	*A. persimilis* Piccinelli & Prosdocimi, 1968: southern South America
Phylum: Arthropoda	*A. urmiana* Günther, 1899: Iran (Urmia Lake; West Azerbaijan Province)
Subphylum: Crustacea Brünnich, 1772	*A. franciscana* Kellogg, 1906: America, Caribbean and Pacific islands
Class: Branchiopoda Latreille, 1817	*A. franciscana monica* Verrill, 1869: USA (Mono Lake; California)
Subclass: Sarsostraca Tasch, 1969	*A. sinica* Cai, 1989: Central and Eastern Asia
Order: Anostraca Sars, 1867	*A. tibetiana* Abatzopoulos, Zhang & Sorgeloos, 1998: China (Tibet)
Family: Artemiidae Grochowski, 1896	*Artemia sp*. Pilla & Beardmore, 1994: Kazakhstan
Genus: *Artemia* Leach, 1819	Parthenogenetic populations of *Artemia*: Europe, Africa, Asia and Australia

2.3.3.2. Ecology and natural distribution

Artemia populations are found largely distributed in about 500 salt lakes and artificial salterns scattered throughout the tropical, sub-tropical and temperate climate zones, along coastlines as well as inland (Triantaphyllidis, Abatzopoulos & Sorgeloos, 1998; Castro, Malpica, Castro, Castro, & De Lara, 2000; Van Stappen, 2002; El-Bermawi, Baxevanis, Abatzopoulos, Van Stappen & Sorgeloos, 2004). These habitats generally have a relatively simple biodiversity, with no other eukaryotes and only a few prokaryotes (Dhont & Van Stappen, 2003; Gajardo & Beardmore, 2012). The distribution of these known sites over the continents is very uneven (Figure 2.4), mainly reflecting sampling and exploitation activities; as such it does not give a precise picture of the actual global occurrence of *Artemia* (Dhont & Van Stappen, 2003). The brine shrimp can survive in environments with a wide salinity range with diverse ionic composition and temperature regimes (Van Stappen, 2011). It has been termed a "survival machine" due to the suite of responses it has evolved to cope with harsh conditions imposed by salt lakes (Gajardo & Beardmore, 2012). Notably among such conditions, is the ability to survive in extreme salinities as low as 3 to as high as 340 g L^{-1} (Post & Youssef, 1977; Gajardo & Beardmore, 2012). No optimum can clearly be defined for salinity of the *Artemia* environment; physiologically however, this optimum must be situated towards the lower end of the salinity range (Van Stappen, 2002). At high salinity extremes, *Artemia* found alive barely manage to survive as their normal physiological and metabolic functions are seriously affected. The lower salinity limit in nature is basically a function of the presence of predatory animals. Although brine shrimp are rarely found in waters with salinity lower than 45 gL^{-1}, physiologically they thrive well in seawater and even in brackish waters (Persoone & Sorgeloos, 1980). With regard to the ionic composition, *Artemia* can withstand environments in which the ratio

of the major anions and cations may be totally different from that of seawater, and even reach extremely high or low values in comparison to natural seawater (Persoone & Sorgeloos, 1980). Depending on the prevailing anions, *Artemia* may thus inhabit chloride, sulphate or carbonate waters or their combinations (Van Stappen, 2002). Apart from salinity, temperature also affects the distribution pattern of *Artemia* (Vanhaecke, Tackaert & Sorgeloos, 1987). No *Artemia* is found in the cold tundra and frost climate types as the year-round prevailing extremely low temperatures preclude *Artemia* development. A large number of strains are found in continental areas with extremely cold winter temperatures, where hot summer temperatures allow the hatching of the cysts and subsequent colonisation of the environment (Van Stappen, 2002). The maximum temperature (strain dependent) that *Artemia* populations tolerate has repeatedly been reported to be close to 35 ˚C, a temperature often attained in the shallow tropical salterns that constitute a large part of the *Artemia* habitats. As for salinity, temperature optima are difficult to define and are also strain dependent; generally, however, the optimum for *Artemia* must be situated in the range 25 - 30 ˚C (Van Stappen, 2002).

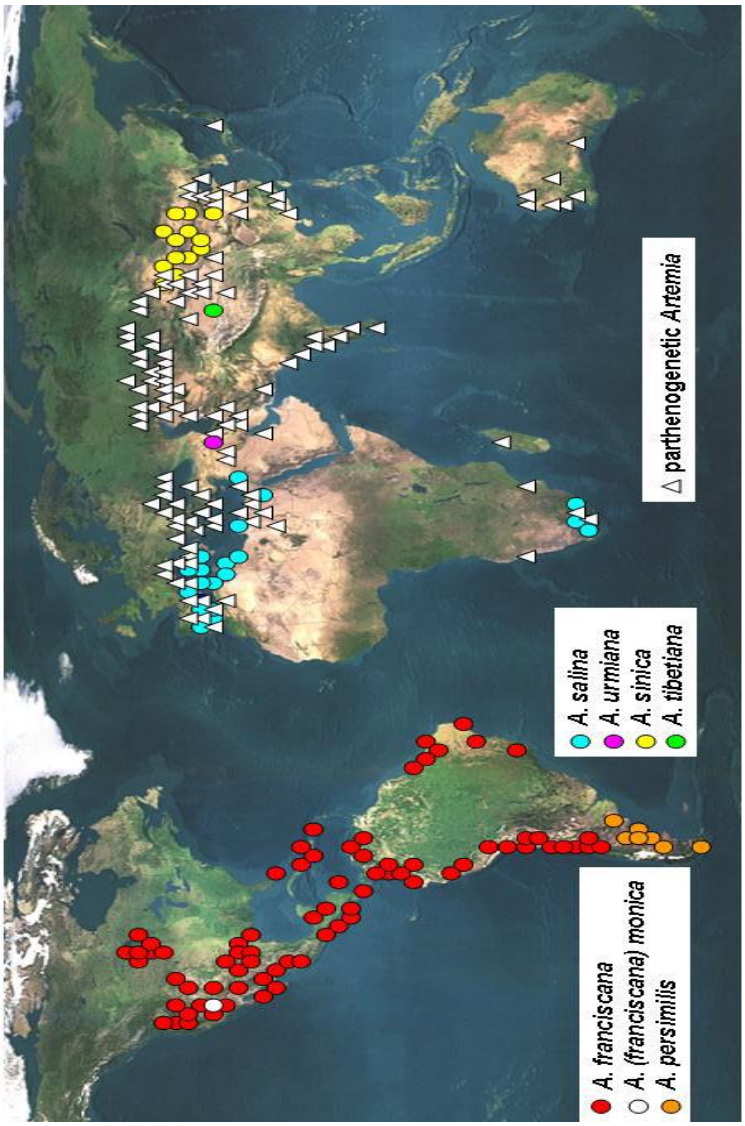

Figure 2.4: Overview of natural *Artemia* sites with known species status
Source: Van Stappen, 2008

2.3.3.3. Life cycle and reproduction

A good account of *Artemia* life cycle and reproductive biology has been described by Van Stappen (1996b) and Criel & Macrae (2002). In optimal conditions, *Artemia* has a longevity of several months, growing from nauplius to adult in just 8 days. Among the many notable characteristics of *Artemia* is the ability to reproduce via two modes switching between ovoviviparity (live birth of nauplii) which occurs usually in favourable environmental conditions, and the alternative oviparity (cyst production) occurring mostly as a response when adverse conditions are anticipated (see Figure 2.5). Fertilised eggs normally develop into free-swimming nauplii (ovoviviparous reproduction) which are released by the mother. In unfavourable conditions (e.g. high salinity, low oxygen levels) the embryos only develop up to the gastrula stage. At this point they are surrounded by a thick shell (secreted by the brown shell glands located in the uterus), enter a state of metabolic dormancy (diapause) and are then released by the female (oviparous reproduction). The alternative mode of reproduction through which the dormant embryos are produced allows for storage of the dried form of the cysts; when sufficiently dried, storage of these cysts can last for a long period even under an extreme temperature range (-273 ˚C to 60 ˚C) without affecting their hatching viability. Contrary to other forms of live food used in larviculture, *Artemia* has been shown to have a high fecundity in which a single female can produce up to 300 nauplii or cysts every 4 days in optimal conditions (Van Stappen, 1996b).

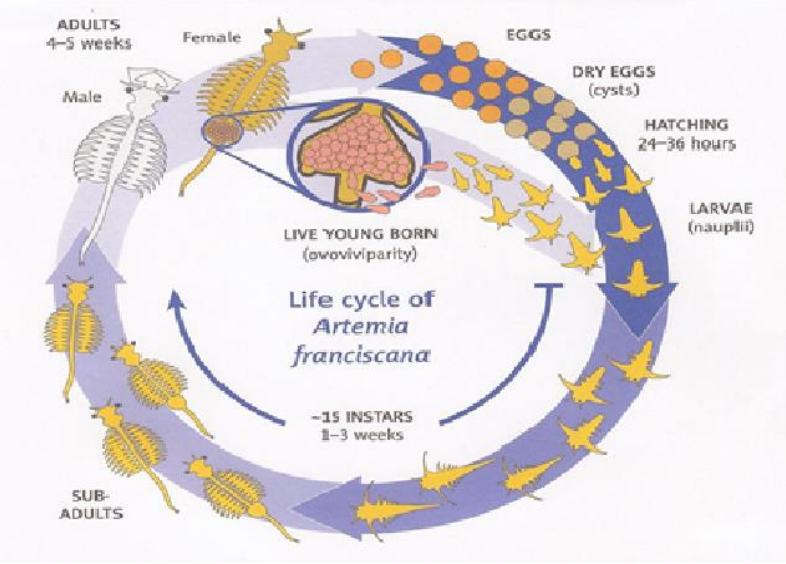

Figure 2.5: The life cycle of *Artemia* displaying two reproductive modes. Source: Ogello, Kembenya, Githukiya, Nyonje & Munguti. 2014

2.4. Nutritional aspects of *Artemia*

2.4.1. Nutritional composition of *Artemia* in general

The gross chemical composition of decapsulated cysts is comparable to freshly-hatched nauplii (Table 2.2).

Table 2.2: Proximate composition (in % of dry matter) of decapsulated *Artemia* cysts and instar I nauplii

	GSL		SFB	
	cysts	nauplii	cysts	nauplii
Protein	± 50	41 - 47	± 57	47 - 54
Lipid	± 14	21 - 23	± 13	16 - 27
Carbohydrate	-	11	-	11
Ash	± 9	10	± 5	6 - 14

GSL, SFB respectively = Great Salt Lake and San Francisco Bay strains of *Artemia franciscana*
Source: Van Stappen (1996a)

Generally, among the nutritional compounds in *Artemia*, the highly unsaturated fatty acids (HUFA) have been found to be beneficial to fish. Specifically, the linoleic 18:2(n-6), linolenic 18:3(n-3), eicosapentaenoic –EPA- (20:5n-3) and docosahexaenoic –DHA- (22:6n-3) acids are of more significance **(**Stottrup & McEvoy, 2002)**.** *Artemia* can be grouped into marine and freshwater types based on their essential fatty acid content (Watanabe, Kitajima & Fujita, 1983). Some *Artemia* strains contain substantial amounts of long chained EPA (20:5n-3) with no, or at most, very low levels of DHA (22:6n-3), which makes them more suitable for marine fish species, while some contain shorter chain linolenic acid (18:3n-3), hence making them suitable for freshwater fish species. In this regard, the choice of the strain to be offered is critical to the survival of the fish larvae especially in the marine species. This is due to the known fact that long chained HUFA are often limiting in marine fishes and they cannot/do not sufficiently biosynthesize them. The required essential fatty acids (EFA) must therefore be supplied in the diet. Through the technique of bioencapsulation, *Artemia* can be enriched with these EFA and other nutrients; and when fed to fish larvae, they serve as vessels for the supply of the limiting nutritional component. Conversely, it is also known that the freshwater fish species can convert the short chained EFA 18:3n-3 into the long chained fatty acids 20:5n-3 and 22:6n-3. On this premise, it is possible to assume that feeding freshwater fish species with *Artemia* strains containing only short chained EFA (those with 18 carbons) should be adequate; using strains possessing long chain EFA would only be of additional advantage. The fatty acid spectrum of cysts and nauplii is nearly identical, although differences can be found in lipid levels, fatty acid methyl ester (FAME) levels, fatty acid composition and energy content of different strains. In addition to availability, decisions on the use of either the decapsulated cysts or the nauplii of *Artemia* should be on the ground of information on their merits and demerits.

2.4.2. Freshly hatched instar I nauplii

The ratio of free amino acids (FAA) to protein content is generally higher for instar I nauplii, compared to cysts, although large variations may exist from strain to strain. This may have dietary consequences when decapsulated cysts are used. In the cysts of brine shrimp, vitamin C is found as ascorbic acid 2-sulfate (AAS) which is a very stable form but with low bio-availability. However, during the hatching process the AAS is hydrolysed into free ascorbic acid, a more unstable form, but directly available in the nauplii for the predator (Van Stappen, 1996a). Certain proteolytic enzymes present in developing embryos and nauplii of *Artemia* are assumed to aid in digestion of the nauplii in the digestive tract of the predator fish (Lavens & Sorgeloos, 1996; El-Dahhar *et al.,* 2013). Contrary to the use of cysts, intensive labour and more hatchery facilities are required when daily hatching is practiced. Since the process of cyst hatching requires energy for breaking of the shell, the hatched nauplii may have low energy available for the larval predator which feeds on them (Van Stappen, 1996a). Also, after the hatching of *Artemia*, empty cysts shells and un-hatched cysts could constitute harvesting problems as well as lead to indigestion when swallowed by fish larvae.

2.4.3. Decapsulated cysts

The use of decapsulated cysts minimizes daily labour, as compared to when nauplii have to be produced oviparously, which requires additional facilities. Furthermore, *Artemia* cysts of a high hatching quality are often expensive, and decapsulation of non-hatching or poorly hatching cysts means valorization of an otherwise inferior product. The cysts have the appearance and the practical advantages of a dry feed and, if they have been dried before application, they have a high floating capacity, and sink only slowly to the bottom of the culture tank. Leaching of nutritional components (as, for example, with artificial diets) does not occur, since the outer cuticular membrane acts as a barrier for larger molecules. On the other hand, since the commercially available *Artemia* cysts are either harvested from natural sources or produced artificially from different biotopes, qualitative variability also exists in the global market. Many factors such as methods of harvesting, processing, storage, environmental changes or type of food available to the parent generations have been implicated to affect cysts resulting in low hatchability and/or poor nutritional composition (Hedayati & Bagheri, 2010; Vasudevan 2012). A correlation was shown between cyclic hydration and dehydration of cysts and a number of quality parameters of cysts and the emerging nauplii (El-Magsodi, Bossier, Sorgeloos, & Van Stappen, 2014). Repeated hydration and dehydration cycles resulted in significantly decreased cyst hatching, reduced starved nauplii longevity and individual energy content and in reduction in vitamin C and fatty acid content.

2.5. *Artemia* production

2.5.1. *Artemia* harvesting at natural production sites

In the early 1960s, commercial availability of *Artemia* cysts was reported from the saltworks in the San Francisco Bay (SFB) area. From the 1970s, a new commercial source of *Artemia* was available from the Great Salt Lake (Utah, USA) with much larger quantities (over 100 tonnes) as compared to SFB (around 10 tonnes) (Hasan, 2016). For many years the Great Salt Lake has been the main source of *Artemia* cysts. However, due to fluctuations in seasonal climatic conditions harvests were variable (Hasan, 2016), but the last few years about 1,000 to 1,500 tonnes of dry Great Salt Lake cyst product have been entering into the market annually. From the 1980s onwards, new sources, be it at small quantities (around 10 tonnes), were available from Australia, Brazil and China. From the mid 1990s, new areas were explored with quantities reaching over 100 tonnes from central Asia (primarily Turkmenistan, Siberia, Kazakhstan). Presently, some 1,500 tonnes of cysts per year enter into the market from countries of central Asia. Also, cyst production from China expanded as several sources were tapped such as the coastal salt pans and inland salt lakes (for example Bohai Bay area and Aibi Lake, respectively), with production reaching over 500 tonnes per year. A significant drop was recorded in recent years where China, being a net exporter of cysts, now imports large quantities, mainly from central Asia (Hasan, 2016). Such drops in production have since caused uncertainty on the sustainability of the natural biotopes to supply enough cysts for aquaculture (Lavens & Sorgeloos, 2000).

2.5.2. *Artemia* production in solar saltworks

Consequent to the fluctuations in the supply of cysts from the natural biotopes, man-managed ponds and salt works have also been used for *Artemia* production worldwide such as in Vietnam, Indonesia, Colombia, Brazil and in some parts of sub-Saharan Africa such as in the East of Kenya. These small-scale production sites provide local developments for local supply of *Artemia* cysts and have contributed to the total marketed cysts product globally. Production of the cysts of *Artemia* in controlled systems has been demonstrated (Royan, Vijayaraghavan, & Krishna Kumari, 1990; Kulasekarapadian & Ravichandran, 2003; Singh & Khandagale, 2006; Litvinenko, Litvinenko, Boiko & Kutsanov, 2015). Cyst production in these solar salt works is practiced either in seasonal or permanent units (Van Stappen, 2011) (Figure 2.6). The seasonal units are usually small in size (of about 100 m2 or more with depths of 0.1 to 0.6 m) and operated for only few months during the dry season. The permanent units consist of several interconnected evaporation ponds and crystallizers where ponds may be a few to several hundreds of hectares with depths of 0.5 – 1.5 m. In both systems sea water is concentrated by evaporation until crystallization, but *Artemia* are found in areas of intermediate salinity (above 80 – 100 g L-1), mainly due to the lack of defence mechanism against predators, which are generally not occurring above this salinity threshold. Optimal water characteristics for commercial production of cysts in salt ponds have been

described. During a 90 days experiment, it was found that up to 63.7 kg ha-1 of cysts could be achieved in salt ponds (6 x 2 m) with water depth of 40 cm, a salinity of 95 g L-1 or higher, a pH of 7.5 or higher, and a water temperature of 33 ˚C or lower (Singh & Khandagale, 2006). Similarly, during a 6 months production season, a pond of 0.7 ha in size with 50 cm depth was inoculated with San Francisco Bay strain of *Artemia franciscana*, and yielded 15 kg of cysts, which is approximately equivalent to 21 kg ha-1 (Royan, Vijayaraghavan & Krishna Kumari, 1990). Animals used in the assessment resorted to oviparity (cysts production) only when salinity went above 140 g L-1.

2.5.3. Tank production of *Artemia*

Indoor production systems of *Artemia* (Figure 2.6) have also been described (Versichele & Sorgeloos, 1980; Lavens & Sorgeloos, 1984; Lavens et al., 1985; Lavens & Sorgeloos, 1987; Lavens & Sorgeloos, 1991). These production systems have only served for niche markets such as the pet markets. However, it is possible to still consider this type of production for self-sustaining local production and use by hatchery owners or for local markets. The abiotic and biotic culture conditions affecting production yields, the design of the culture systems and their operating procedures, disease control, harvesting techniques and production yields, and subsequent utilization of the produced *Artemia* in aquaculture have been described for the production of *Artemia* in super-intensive tank culture systems (Lavens & Sorgeloos, 1991). These authors described different systems which could serve as alternatives to pond production.

For tank production purposes, the use of seawater is the most practical (Dhont & Lavens, 1996; Lavens & Sorgeloos, 1996). Depending on the desired salinity, adjustment of the seawater can be done by adding brine or diluting with tap water (Dhont & Lavens, 1996). With regard to ions, chloride-habitat *Artemia* populations require Ca^{2+} concentrations higher than 20 mg L^{-1} whereas carbonate-habitat strains prefer concentrations lower than 10 mg L^{-1} in combination with low levels of Mg^{2+}. In general, diluting saline culture water with freshwater before usage could be helpful in the maintenance of the ionic composition (Dhont & Lavens, 1996). Culture temperature is strain specific but for most strains a common range of preference is 19-25 ˚C. The generally accepted pH tolerance for *Artemia* ranges from 6.5 - 8. Regarding oxygen, only low concentrations of less than 2 mg L^{-1} will limit the production of biomass. Optimally however, oxygen concentrations higher than 2.5 mg L^{-1} are suggested. As for ammonia, nitrite and nitrate, the tolerance level for *Artemia* is considered very high as no significant effect has been found on survival and growth at concentrations up to 1,000 mg L^{-1} and 320 mg L^{-1} for NH_4^+ and NO_2^- - N respectively (Dhont & Lavens, 1996).

In spite of the technical feasibility, authors however have also expressed concern with the cost of production as a function of high operational sophistication and high energy and feed demands when such tank systems are used. Techniques such as the use of cyclic low oxygen tension and the addition of ferric-EDTA which stimulates the synthesis of haemoglobin with subsequent shift of mode of reproduction from ovoviviparity to oviparity have been demonstrated (Versichele & Sorgeloos, 1980). Controlled production of cysts was also described using cyclic flushes of the cultures with N_2-gas as induction mechanism for oviparity (Lavens & Sorgeloos, 1984).

Figure 2.6: Indoor intensive *Artemia* production system (left) and an outdoor *Artemia* cysts production in Bohai Bay saltponds (right, source: Sui & Xin, 2016)

2.6. Controlled production of ovoviviparous *Artemia* nauplii

2.6.1. High oxygen levels and low salinity

To our knowledge, only the work of Lavens and Sorgeloos (1987) described the technique for conditioning *Artemia* biomass for the purpose of ovoviviparous nauplii production. This technique involves maintaining high oxygen levels, optimal culture water exchange and diet manipulation. Using this technique, a yield of up to 30 g wet weight nauplii day^{-1} 100 L^{-1} culture tank was achieved. Generally, oxygen and salinity are considered among the most important abiotic factors in *Artemia* culture. Using a flow-through culture system consisting of 100 L culture tanks with oxygen levels kept around 4 mg L^{-1}, the above authors showed the possibility of maintaining a high density biomass (5,000 brine shrimps L^{-1}) of 2 weeks old over a period of 3 weeks with exclusive ovoviviparous nauplii production. Contrary to the cyclic oxygen stress technique used in previous studies to induce and maintain oviparous reproduction (Lavens and Sorgeloos, 1984; Berthelemy-Okazaki and Hedgecock, 1987), the high constant oxygen levels were used as a means to minimize stresses for the purpose of achieving ovoviviparity by these authors.

With respect to salinity, the authors conducted their study using artificial seawater of 50 g L^{-1} salinity. However, it is known that in aquatic animals, osmoregulation occurs with a high caloric cost as salinity increases. When caloric cost of osmoregulation increases, energy meant for

supporting other physiological functions is consequently depleted (Wedemeyer, 1996). This results in stress, which in *Artemia* influences its reproductive mode by switching to oviparity. It is for this reason that *Artemia* cyst production has generally been conducted at high salinities that are well above the sea water level (Versichele & Sorgeloos, 1980; Lavens & Sorgeloos, 1984; Kulasekarapadian & Ravichandran, 2003; Litvinenko, Litvinenko, Boiko & Kutsanov, 2015). On the other hand, *Artemia* thrive well in sea water salinity and even in brackish water at 10 g L^{-1} salinity (Persoone & Sorgeloos, 1980; Van Stappen, 1996b; Van Stappen, 2011). Despite that the lower salinity threshold for *Artemia* in nature is determined by the salinity tolerance of its predators, it has been reported that a parthenogenetic population exists in small temporary lagoons around Lake Urmia, which reaches maturity at 10 gL^{-1} and reproduces within the salinity range 15-20 g L^{-1} (Agh *et al*., 2007). Studies have shown that some strains of *Artemia* respond better at low salinity. Vanhaecke, Siddall and Sorgeloos (1984) reported that different strains of *Artemia* could survive over a wide range of salinity (35 – 110 g L^{-1}), but significant differences were revealed by some strains in their response to the lower end (5 g L^{-1}) of the salinity range tested with considerably good survival recorded. A study by Soundarapandian and Saravanakumar (2009) also reported the culture of two strains belonging to parthenogenetic and bisexual populations at low salinity range, between 20 and 35 g L^{-1}. Hence, assessing ovoviviparity at salinity levels lower than the 50 g L^{-1} used by Lavens & Sorgeloos (1987), aiming to reduce stressful culture conditions, could yield interesting results.

2.6.2. Optimal water exchange

Optimal culture water exchange was also considered as a factor for inducing ovoviviparity in *Artemia*. Quick removal of soluble and particulate waste minimizes fluctuations in the culturing conditions. This was achieved by maintaining a high flow rate of 100 L h^{-1} with a retention time of 1 h (Lavens & Sorgeloos, 1987). Although of secondary importance, it also contributed to the eventual harvest of freshly-released nauplii.

2.6.3. Diet manipulation

Good results were achieved with a mixed diet of fresh baker's yeast and an emulsified enrichment product of HUFA and vitamins, occasionally supplementing with a corn by-product. The lipid enrichment did not only enhance fecundity levels, but also ensured a high nutritional quality of the offspring. The baker's yeast has optimal physical properties i.e. maximal ingestibility (single cell protein), minimal probability of bacterial breakdown; also, its deficiency in pigments was assumed to cause an incapability in the *Artemia* to produce the haem-pigment which is an essential component in the oviparous reproduction mode (Lavens & Sorgeloos, 1987). Several alternative feed sources have been successfully tested in *Artemia* indoors culture. Dobbeleir *et al.* (1980) listed some which include dried algae such as *Chlorella, Scenedesmus, Spirulina,* yeasts and

various inert products such as fish meal, egg yolk and homogenized liver. Coconut meal and sugarcane molasses have also been successfully used (Talloen, 1978; Dobbeleir *et al.*, 1980). Considering cost and availability, agricultural crops constituting mainly grains and their by-products such as rice bran (Sorgeloos, Baeza-Mesa, Bossuyt, Bruggeman, Dobbeleir, Vaersichele, Lavina and Bernardino, 1980) and corn by-product (Lavens and Sorgeloos, 1987) have also been successfully utilized for rearing of *Artemia* biomass in indoor conditions. Although these feed sources could influence the cultured biomass, however, their use as sole diets for sustaining an actively reproducing population over several generations still remains to be verified.

2.7. The African catfish (*Clarias gariepinus*, Burchell, 1822)

2.7.1. Biology of *Clarias gariepinus*

2.7.1.1. Morphology and taxonomy

Taxonomically, the fish species has been classified as belonging to:

Domain: Eukaryota
Kingdom: Metazoa
Phylum: Chordata
Subphylum: Vertebrata
Class: Actinopterygii
Order: Siluriformes
Family: Clariidae
Genus: Clarias
Species: *Clarias gariepinus*

“Morphologically, it has a large depressed head which is completely encased above with a large sub-terminal mouth, and a narrow body which is strongly compressed towards the caudal part (Figure 2.7). It varies in colour from sandy-yellow through grey to olive with dark greenish-brown markings and a white belly. Size and age at maturity vary greatly (150 - 750 mm total length = TL; between one and four years) although the average size at sexual maturity is around 300 - 350 mm TL. The species is equipped to feed on a variety of food organisms ranging from phytoplankton to fish. The mouth is wide, sub-terminal and transverse. The buccal cavity is capable of considerable vertical displacement that enables suction feeding. The stomach is muscular, and the intestine is thin walled and relatively short, implying a dependence on high protein foods” (Hecht, 2013b).

Figure 2.7: Adult *Clarias gariepinus.* Source: Hecht (2013b)

2.7.1.2. Ecology and natural distribution

“Of all freshwater fishes, it is the species with the widest latitudinal range in the world (about 70 degrees latitude). The species has almost a pan-African distribution ranging from the Gariep (Orange) River in South Africa northwards through central, west and north Africa; through the Middle East and into eastern Europe. They are found in lakes, streams, rivers, swamps and floodplains. The most common habitats are floodplains, swamps and pools” (Hecht, 2013b).

2.7.1.3. Life cycle and reproduction

“Clarias gariepinus undertake lateral migrations from larger water bodies, in which they feed and mature at about the age of 12 months, to temporarily flooded marginal areas in order to breed. Final gonadal maturation is associated with rising water levels. Under stable environmental conditions, adult *C. gariepinus* have mature gonads year-round. In ideal conditions, a ripe female may lay about 60,000 eggs kg^{-1}. Eggs hatch in 20 – 60 h depending on temperature. Sexual differentiation begins between 10 and 15 days after hatching. As flooded marginal areas dry up with the end of the rains, juveniles and adults make their way back to the deeper water where they complete their growth to adult size. Growth is relatively rapid, approaching maximum size within a couple of years. The first year growth is nearly linear resulting in the large initial jump in size” (Hecht, 2013b).

2.7.2. Production systems

Traditional flooded ponds, where naturally recruited fingerlings are gathered during the rainy season and exogenous feed is provided, constitute a form of capture-based aquaculture in many countries. More recently, with the development of balanced feed, there has been a huge diversification of culture environment in most African countries where catfish are reared, including the use of concrete or fibre glass tanks and water recirculation systems. Other on-growing techniques used in Africa and many other catfish producing countries include catfish holes as practiced in Bangladesh and Nepal, polyculture ponds in earthen ponds, tanks, raceways and cages. Currently, the total production in sub-Saharan Africa is estimated at 229,090 tonnes (FAO, 2017).

2.7.3. Larviculture procedures

In nature, the African catfish reproduces in response to environmental stimuli such as a rise in water level and inundation of low-lying areas. These events do not occur in captivity and hormone treatment is employed to ensure large-scale production of catfish fingerlings. The hormones used include ovaprim, deoxycorticosteroneacetate (DOCA), human chorionic gonadotropin (HCG), pituitary glands from catfish brooders, common carp, Nile tilapia and even frogs, following special technical procedures. Average relative fecundity is in the region of 20,000 - 25,000 eggs kg^{-1} body

weight. Egg and larval development are rapid. Depending on temperature (with an optimum of 28 ^{o}C) the larvae hatch after 24 - 48 h. The embryonic development of *C. gariepinus* was studied from the oocyte activation to the end of endogenous feeding (164 h post-fertilization) (Osman, Wertz, Mekkawy, Verreth & Kirschbaum, 2008). This study found that hatchlings possessed an undifferentiated digestive system, and differentiation of buccal cavity, oesophagus and intestine was only recorded at 96 h (four days) post-fertilization. The openings between these parts of the digestive tract as well as the formation of the intestine valve and epithelium folds only occurred at 144 h (six days) post-fertilization. Also, these authors found that the eye differentiation was concomitant with first feeding which might suggest a key role of visual stimuli in feeding behaviour. A similar study by Olaniyi and Omitogun (2013) showed that a fully vascularised buccal cavity and well developed digestive system became functional only after day 4 post-hatch. The eleutheron-embryonal stages and digestive canal of *C. gariepinus* at 144 h post-fertilization are illustrated below (Figure 2.8 and 2.9, respectively).

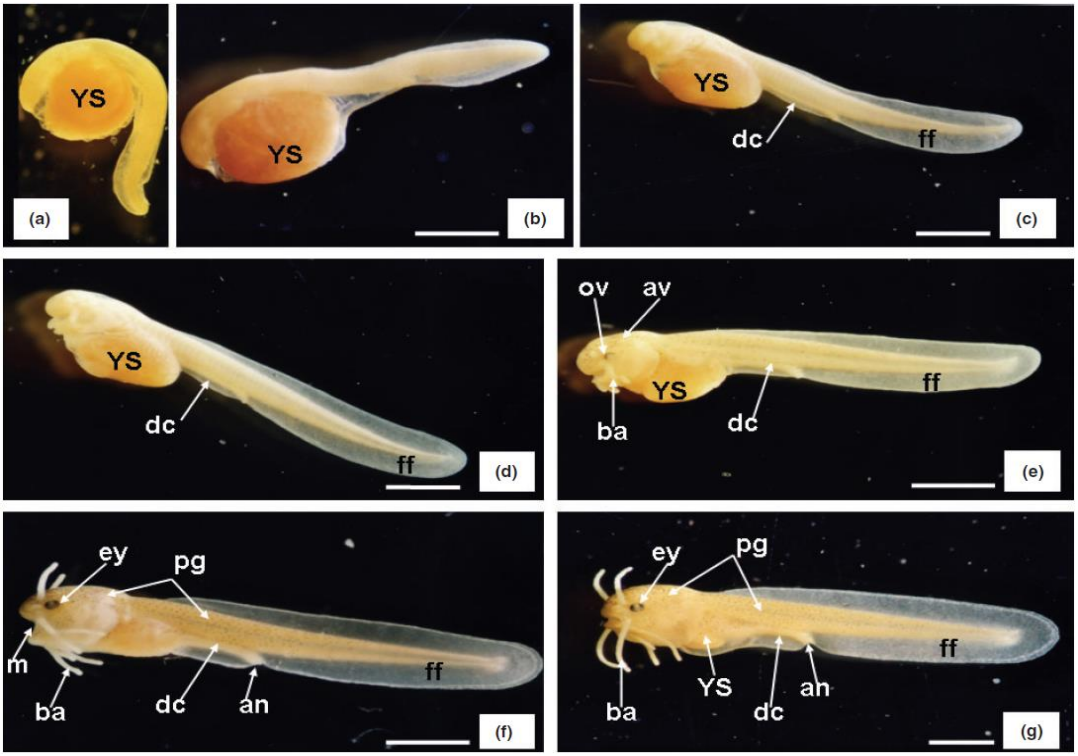

Figure 2.8: Eleutheron-embryonal stages of *Clarias gariepinus* showing (a) newly-hatched embryo (40 h-PF), (b) 48 h-PF, (c) 72 h-PF, (d) 96 h-PF, (e) 120 h-PF, (f) 144 h-PF, (g) 164 h-PF. ys = yolk sac, ff = fin fold, dc = digestive canal, av = auditory vesicle, op = optic vesicle, ba = barbels, m = mouth, ey = eye, pg = pigments, an = anus, PF = post-fertilization. Scale bar = 1 mm. Source: Osman *et al.* (2008)

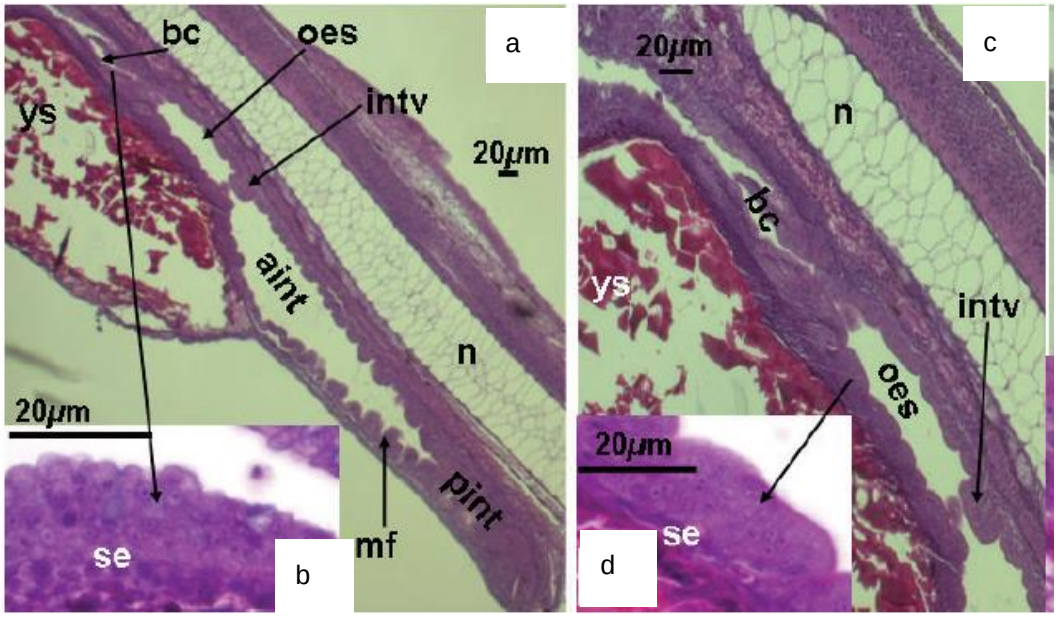

Figure 2.9: a and c = Digestive canal of *Clarias gariepinus* larvae 144 h (6 days) post-fertilization at 25 ˚C; b and d = squamous epithelium, bc = buccal cavity, oes = oesophagus, int = intestine, intv = intestinal valve, aint = anterior intestine, pint = posterior intestine, mf = mucosal fold, se = squamous epithelium, n = notochord, ys = yolk sac. Source: Osman *et al*. (2008)

The yolk sac is absorbed within 3 – 4 days and the stomach is fully functional within 5 – 6 days after the onset of exogenous feeding. Exogenous feeding commences within 80 h of hatching and the larval period lasts for 7-10 days. Regarding the propagation technique and quality of feed used in the culture of this species in Africa, Hecht (2013a) classified them into extensive and intensive systems. In the extensive hatcheries, larvae are fed with a mixture of cow brain in addition to egg yolk just after vitelline resorption for 4 - 6 days before stocking in nursing ponds. In such systems survival averages 25 – 35 % with low growth rates even in properly managed systems. On the contrary, in intensive systems, larvae are fed with live food such as *Artemia* and up to 75 % survival is recorded. These hatcheries tend to offer good quality fingerlings for on-growing by fish farmers. Indeed, several studies have shown that live food in the form of *Artemia* nauplii, small *Daphnia, Moina* or rotifers are essential for the first 4 - 6 days after which they are gradually weaned onto a dry diet from 6 - 7 days to the end of day 10. Although *C. gariepinus* larvae possess a well advanced digestive system at the onset of exogenous feeding, they lack a functional stomach and consequently pepsin digestion (Verreth, Eding, Rao, Husken, & Segner, 1993); these authors asserted that this could be related to the requirement for live food by this species. The dietary preferences of *C. gariepinus*, indicating the dependence of the larvae on the zooplankton, have been illustrated by Hecht (2013b) (Table 2.3).

Table 2.3: Dietary preferences of African catfish *Clarias gariepinus* by length group

	Life stage/size class					
Type of food	Larvae	Fry	Fingerling	Juvenile	Sub adult and adult	Large adult
		20-50 mmTL	50-100 mmTL	100–300 mmTL	300–700 mmTL	>700 mmTL
Zooplankton	100%	*****	*	*	*	
Insects		***	****	***	**	
Molluscs		**	***	**	**	
Benthic Crustaceans		*****	***	****	****	
Detritus		*	*	*	*	
Algae			*	*	*	
Fish			***	*****	*****	*****
Surface scum					*	
Phytoplankton					*	
Macrophytes					*	

TL = total length. Number of asterisks (*) signifies degree of diet preference. Note: Larvae (column 2) preference on 100 % zooplankton. Source: Hecht (2013b)

2.7.4. Nutritional requirements of *Clarias gariepinus* larvae

Although the nutritional requirements of *C. gariepinus* larvae are not fully known, some recommendations have been proposed (Hecht, 2013a) which appear to promote optimal growth and body condition. The larvae require a diet consisting of 55 % crude protein, 2.5 % methionine, 9 % crude lipid and 21 % carbohydrate. Except for methionine, the quantitative requirements of other amino acids are not known. Verreth (1994) found that the eggs of African catfish contain moderate amounts of lipid of a high polar nature of which 70 – 75 % consist of phosphatidylcholine, which is an important energy source during the early life stages of the species. When feeding *Artemia* to marine fish larvae, it is generally known that highly unsaturated fatty acids (HUFA) such as eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) are of more significance as they determine the nutritional value for the fish larvae. It is also known that unlike marine fish, freshwater fish generally have the capacity to biosynthesize these essential fatty acids using short chain fatty acids such as linolenic acid (18:3n-3). The biosynthesis of the n-3 fatty acids starts from the yolk sac period onwards; hence feeding of *C. gariepinus* larvae with n-3 HUFA enriched diet does not influence growth and survival but rather affects liver ultra structure (Verreth, 1994).

In an attempt to improve the performance of African catfish larvae in aquaculture, studies such as those of Verreth, Storch and Segner (1987); Kerdchuen and Legendre (1994); Chepkirui-Boit, Ngugi, Bowman, Oyoo-Okoth, Rasowo, Mugo-Bundi and Cherop (2011); Enyidi, Pirhonen and Vielma (2014) have been undertaken showing the critical period during which they require live food and the food type that will result in optimum yield. Several of such studies have shown the importance of live food (*Artemia* nauplii) particularly in the larval stage of the fish species. Overall, Enyidi *et al.* (2014) highlighted that larval *C. gariepinus* requires live food at the onset of

exogenous feeding after which dry feed can be administered. Verreth (1994) also asserted that in order to wean the African catfish larvae successfully to a dry diet without loss of growth, the larvae should be fed live food organisms during the first four days of exogenous feeding.

Chapter 3

Survival, reproductive and life trait responses of four *Artemia* strains in low salinity

CHAPTER 3

SURVIVAL, REPRODUCTIVE AND LIFE TRAIT RESPONSES OF FOUR *ARTEMIA* STRAINS IN LOW SALINITY

Abstract

In the wild, the brine shrimp *Artemia* is adapted to varying saline environments ranging from 3 g L^{-1} to 340 g L^{-1}. Their existence in high salinity environments is strategic, mainly to avoid predators. Otherwise, they thrive well at sea water salinity. Despite the adaptation to high salinity, *Artemia* becomes physiologically stressed as the environmental salinity increases, leading to mortality and switching of their mode of reproduction from the normal ovoviviparity (live birth) to the alternative oviparity (cysts production). Contrary to the usual practice of culturing in high salinity for the purpose of cyst production, low salinity ranging from 0 – 32 g L^{-1} was used in this study to test the effect on survival and some reproductive and life traits of four *Artemia* strains, consisting of two bisexual strains (the Great Salt Lake and Vinh Chau *Artemia franciscana*) and two parthenogenetic (Tuz and Balikun) strains. Survival was tested in axenic and xenic conditions. In the axenic condition, instar I and II developmental stages (choice of developmental stages was based on the concept that environmental salinity does not influence embryo and instar I of *Artemia*) of all the four strains were tested over a 48 h period. In the xenic condition, two inoculation ages i.e. 0 days and 8 days old animals, representing freshly hatched nauplii and pre-adults (based on the concept that mortality in *Artemia* reduces at maturity) was tested over a period of nine days. The reproductive and life traits of the different *Artemia* strains were also assessed xenically in the respective salinities. The results generally showed that salinity had an influence on survival with respect to the strain types in the axenic and xenic conditions; whereas both salinity and strain types influenced the reproductive and life traits measured. Total mortality occurred at 0 g L^{-1} (freshwater) while lowest survival occurred at 5 g L^{-1} in both conditions. In the xenic test, higher survival of 97.8 ± 2.1 and 98.0 ± 2.0 % at 20 and 32 g L^{-1}, respectively, were observed with the GSL strain ($P<0.05$). No difference ($P>0.05$) was found between the two instar developmental stages tested. Similarly, no differences ($P>0.05$) were observed between the inoculation ages at the different salinities except at 5 g L^{-1} where the lowest survival was recorded. Overall best performance in terms of the reproductive and life traits was observed with the GSL strain. In particular, the GSL strain had the highest total offspring as well as the highest offspring produced as nauplii ($P<0.05$). We conclude that 20 g L^{-1} salinity and the GSL strain are the most suitable for application in the mass culture.

3.1. Introduction

Artemia populations existing naturally in the wild inhabit environments with a wide range of salinity. *Artemia* have been found in salinities as low as 3 g L^{-1} and as high as 340 g L^{-1} (Gajardo & Beardmore, 2012). Consequently, their culture either in large artificial ponds for commercial purposes or in the laboratory for experimental purposes has also been conducted in media at varying levels of salt concentrations. Several investigations, particularly in terms of survival and growth characteristics have been conducted at ranges between sea water salinity to levels which are well above it (Browne & Wanigasekera, 2000; Abatzopoulos, El-Bermewi, Vasdekis, Baxevanis & Sorgeloos, 2003; El- Bermawi *et al.*, 2004; Agh *et al*., 2008; Castro-Mejia, Castro-Barrera, Hernandez, Arredondo-Figueroa, Castro-Mejia & De Lara-Andrade, 2011; Aalamifar, Agh, Malekzadeh & Aalinezhad, 2014). Generally, *Artemia* perform efficiently at a salinity range 30 – 80 g L^{-1} (Lavens & Sorgeloos, 1991). Physiologically, *Artemia* has been regarded to thrive well at sea water salinity (Van Stappen, 1996a). Considering that high caloric cost for osmoregulation occurs at higher salinity (Wedemeyer, 1996), other physiological functions will consequently occur less optimally and conversely, better physiological performances are expected to occur at low salinity.

Studies have shown that certain strains, however, thrive better at low salinity. During a nine day study for the combined effects of temperature and salinity on the survival of *Artemia* of various geographical origin, high survival was recorded at salinities ranging from 35 to 110 g L^{-1} in twelve out of thirteen strains tested. Significant differences in response to lower salinities between 5 and 15 g L^{-1} revealed that some strains such as the *Artemia persimilis* strain from Buenos Aires, Argentina, could perform considerably better than the others tested (Vanhaecke *et al*., 1984). A laboratory study using parthenogenetic and bisexual *Artemia* from India (exact strains not specified) cultured over 20 days in freshwater (2 g L^{-1}), brackishwater (28 – 33 g L^{-1}) and seawater (34 – 55 g L^{-1}) showed that high survival of both strains was achievable with the brackishwater (75 % survival) and seawater (80 % survival), but survival was low in the freshwater (Soundarapadian & Saravanakumar, 2009). A parthenogenetic population in small temporary lagoons around Lake Urmia, Iran, was monitored in the field over a period of two years, and was observed to reach maturity at very low salinity of 10 g L^{-1}, and to reproduce at salinity between 15 - 20 g L^{-1} (Agh *et al*., 2007).

Within the overall scope of this research work, the culture of *Artemia* biomass for the purpose of nauplii production by utilizing the ovoviviparous reproductive mode was tested. Nauplii production using ovoviviparously reproducing biomass has previously been described, using a technique which involves diet manipulation, optimal culture water exchange and high constant oxygen levels (Lavens & Sorgeloos, 1987). The culture was conducted at 50 g L^{-1} salinity and the system yielded up to 30 g wet weight nauplii day^{-1} 100 L^{-1} culture tank at a stocking density of 5,000 adults L^{-1} over

a culture period of 35 days. Therefore, the present study further considered culturing *Artemia* at low salinity. Contrary to the practice of culturing in high salinity for the purpose of cyst production (oviparity), low salinity was considered to support good physiological condition, and consequently to result in high survival and ovoviviparity.

In addition to the low salinity treatment and in order to ensure high survival is achieved during culture, the test also considered the responses of *Artemia* at some selected growth stages. It has been reported that different growth stages in *Artemia* are affected differently by salinity (Clegg & Trotman, 2002). According to these authors, the enzyme sodium potassium adenosine triphosphatase (Na, K-ATPase) is known to be involved with the osmoregulatory mechanism in both nauplii and adult stages of *Artemia*. However, the level of the enzyme found in the nauplii is based on a programmed mechanism which is independent of environmental salinity. This programmed mechanism has been considered to occur only for a short-term, mainly from the period of emergence of the embryo to the first nauplii development stage. In contrast to the apparently independent osmoregulatory mechanism of the nauplii, the Na, K-ATPase in adult *Artemia* seems to be influenced by the environmental salinity (Clegg & Trotman, 2002). Conversely, Vanhaecke *et al*. (1984) asserted that mortality rate in *Artemia* reduces as they grow to adult stages, while other studies (Wear and Haslett, 1986; Wear, Haslett and Alexander, 1986) reported that adult *Artemia* are more tolerant than juveniles. On these premises, two hypotheses were formulated. Firstly, we assumed that if the independent osmoregulatory mechanism in the nauplii is limited only to the instar I stage (Clegg and Trotman, 2002), then there is a likelihood of increased mortality rate when the instar II stage is used for inoculation of culture. Secondly, that if mortality rate reduces in adult *Artemia* and if the adults are more tolerant than the juveniles (Vanhaecke *et al*., 1984; Wear *et al.,* 1986), then it will be more benefial to inoculate the culture with the adults rather than with the nauplii.

As salinity preference and tolerance in *Artemia* may be strain-specific and influenced by growth stages respectively, prior to setting up a mass culture, laboratory tests were conducted with the aim to select a suitable low salinity level, an *Artemia* strain with high rates of survival and ovoviviparity, and an appropriate inoculation age for subsequent application in *Artemia* mass culture. The objective was to investigate the effect of low salinity on the survival of a number of *Artemia* strains at different growth stages, and on their reproduction and life traits.

3.2. Materials and methods

3.2.1. *Artemia* strains used

Two strains belonging to the species *Artemia franciscana* (Kellogg, 1906) and two parthenogenetic strains were used in the study (Table 3.1). Cysts of all four strains were obtained from the cyst

bank of the Laboratory of Aquaculture & *Artemia* Reference Center (ARC), Ghent University, Belgium. The cysts had been under storage in air tight packs under cold conditions (+4 ˚C).

Table 3.1: Species/strain, origin, reference number, mode of reproduction and abbreviations of *Artemia* used in the study

Species/Strain	Country of origin	ARC reference number	Reproductive mode	Abbreviations Used
A. franciscana/Great Salt Lake	USA	1768	B[1]	GSL
A. franciscana/VinhChau	Vietnam	1787	B	VC
Parthenogenetic/Tuz	Kazakhstan	1761	P[1]	TUZ
Parthenogenetic/Balikun	China	1781	P[1]	BLK

B= Bisexual; P= Parthenogenetic; 1=Van Stappen (2002)

3.2.2. Cyst diameter measurements of the four *Artemia* strains

The standard procedure for measuring hydrated cysts was used (Vanhaecke & Sorgeloos, 1980a). A small sample of cysts for each of the strains was hydrated in medium of 10 g L^{-1} salinity for 1 h after which 1 % lugol was added and they were incubated for another 2 h. Thereafter, 1 % lugol was again added before cysts were transferred into a 14 mL Falcon tube filled to the 7 mL mark and kept overnight in the dark. The diameter of 100 cysts of each strain was measured with the binocular microscope after calibration with a stage plate and an ocular micrometer to obtain the calibration factor.

3.2.3. Experimental design

3.2.3.1. Overview of experiments

Three different experiments were conducted, testing for the performance of *Artemia* at low salinities (i.e. in the range 0 to 32 g L^{-1}). In Experiment 1 and 2, *Artemia* were hatched in standard seawater salinity (32 g L^{-1}), and incubated into lower salinity at different developmental stages: in experiment 1 these were instar I and instar II; in Experiment 2 these were instar I and 8 days-old pre-adults. In both cases survival was recorded. In Experiment 3, animals from Experiment 2 (those exposed to low salinity from instar I onwards), after termination of the previous test, were taken for subsequent observation of their reproductive behaviour at low salinity.

3.2.3.2. Effect of low salinity on the survival of instar I and II developmental stages of the different strains in axenic conditions (Experiment 1)

The survival of nauplii of the different *Artemia* strains at instar I and II developmental stages was tested at 0, 5, 10 and 32 g L^{-1} salinity over a 48 h experimental period, whereby the highest salinity

was used as control. The different culture media (except 0 g L^{-1} which was fresh municipal water) were prepared by mixing natural sea water of 32 g L^{-1} salinity with fresh municipal water and the desired salinity was checked with a digital refractometer (Hanna Instruments: HI 96822) to the nearest 1 g L^{-1}.

The test was conducted axenically (Baruah, Ranjan, Sorgeloos & Bossier, 2010). In order to maintain axenicity of cysts and nauplii, all manipulations were performed under a laminar flow hood (Figure 3.1). Sterilized Falcon tubes (FT) were used for hydrating the cysts and aeration was supplied through disinfected (using a solution of ethanol and denaturol) air tubes fitted with sterilized air filters and connected to an external air source. To neutralize the disinfectant, air was allowed to pass through the tubes for a few minutes. Air tubes were then inserted to the bottom of the FTs containing 18 mL distilled water, to ensure cysts were suspended within the water column during hydration and decapsulation. For each strain a small quantity of cysts (0.2 g) was added into the FTs and was allowed 1 h hydration period to ensure adequate decapsulation. In order to prevent pH from dropping below the desirable range, 660 µL NaOH was added into each FT while cysts were still being aerated. Subsequently, 10 mL NaOCl was added to decapsulate the cysts. Approximately 2 min was allowed until cysts colour changed to orange, after which 14 mL sodium thiosulphate ($Na_2S_2O_3$) was added to neutralise the hypochlorite. At this point, cysts were sieved over a filter of 50 µm mesh size and thoroughly rinsed with 35 g L^{-1} filtered autoclaved sea water (FASW). Rinsed decapsulated cysts of each strain were transferred into two FTs filled with FASW for hatching. The FTs were placed on a rotor (to ensure cysts were kept in continuous suspension) at a rotating speed of 5 rpm in a temperature controlled room (28 ˚C) and exposed to a constant illumination of approximately 27 μEm^{-2}.sec (Figure 3.1).

The FTs were checked regularly from 16 h of incubation onwards for appearance of first instar nauplii. One FT per strain was retrieved from the rotor at the sight of adequate amounts of free swimming nauplii. Batches of small amounts of instar I were taken and rinsed with autoclaved solutions of the different salinity concentrations over a 150 µm sterile filter before the nauplii were transferred into sterile petri dishes containing medium of the same salinity. Twenty individuals each were pipetted under the binocular microscope from the respective petri dishes into triplicate autoclaved screw cap bottles containing 20 mL of the respective experimental salinities, which was adjusted to 30 mL final volume. Feeding was done once with autoclaved *Aeromonas harveyi* (LVS3) at 10^7 CFU mL^{-1} before bottles were tightly covered and placed randomly on a rotor in the temperature controlled room as described above. LVS3 was prepared according to standard procedures (Defoirdt *et al*., 2006) and the density was determined using the following equation:

$$\text{Concentration } (CFUmL^{-1}) = [1200 \times 10^6 \times OD] \times d$$

Where: OD = optical density; d=dilution factor

The second batch of FTs containing the different strains was retrieved from the rotor approximately 8 h later, which is the approximate period for molting of nauplii from instar I to II (Van Stappen, 1996b). All further experimental procedures were exactly as described for instar I above. Survival of each batch was scored at the end of 48 h. This was achieved by pouring the entire content of each FT into a petri dish and counting surviving animals under the binocular microscope.

Figure 3.1: A session under the laminar flow hood during the decapsulation of the *Artemia* cysts and inoculation of nauplii into culture tubes (left picture); screw cap tubes containing instar I and II nauplii mounted on a rotor in a temperature controlled room (right picture)

3.2.3.3. Effect of low salinity on the survival of instar I and pre-adults in xenic conditions (Experiment 2)

Artemia were incubated in low salinity medium at two different ages (i.e. at 0 days representing freshly hatched nauplii and at 8 days representing the pre-adult stage) and their survival was recorded over a period of nine days. The different salinities (0, 5, 10, 20 and 32 g L^{-1}, with the highest salinity used as control) were prepared and checked as described in the axenic test (see 3.2.3.1). The test was conducted xenically. Cysts were incubated and hatched following standard procedures as described by Sorgeloos, Lavens, Le'ger, Tackaert & Versichele, (1986). Experimental procedures were adapted from Vanhaecke *et al.* (1984). For the animals incubated into low salinity at 0 days, 50 freshly hatched nauplii for each of the different strains were pipetted under the binocular microscope into triplicates of 500 mL bottles filled to the 300 mL mark with the different experimental saline media. Animals in each bottle were immediately fed 2 mL (at a concentration of approximately 27.5 million cells mL^{-1}) fresh *Tetraselmis suecica* algae and subsequently feeding was done *ad libitum* based on visual observation. Bottles containing nauplii were placed randomly into a water bath in a temperature (28 °C) controlled room and aeration was supplied using tubes inserted into each of the bottles (Figure 3.2). Illumination (2,000 lux) was provided using fluorescent tubes. Every third day, culture water was changed with freshly diluted water of the same salinity and survival was scored after nine days. This was done by gently pouring the entire content of the glass vessel through a 100 µm mesh filter; trapped animals were immediately rinsed into a petri dish containing freshly prepared medium of the same salinity.

Culture bottles and filter were carefully checked and properly rinsed to ensure no animal was lost. Animals were then pipetted back into the bottles and were fed and returned to the water bath. As for the animals incubated at the age of 8 days, a portion from the same batch of hatched nauplii, used for the 0-day incubation treatment, was reared for eight days in 32 g L^{-1} sea water before they were inoculated into the different experimental salinities. All further procedures were exactly as described for the 0-day incubation treatment.

Figure 3.2: Culture bottles containing *Artemia* at incubation ages 0 and 8 days, placed in water bath during the xenic test

3.2.3.4. Effect of low salinity on reproductive and life traits of the different *Artemia* strains (Experiment 3)

Reproductive and life traits of the different *Artemia* strains were assessed at the five low salinities used in the xenic test (Experiment 2). The group of animals which were incubated in low salinity at the nauplius stage (0 days incubation age) and grown to pre-adults in the xenic survival test were pooled according to the different salinity treatments after the nine days test period, and were used for further monitoring of their reproduction. Experimental procedures were adapted according to Browne and Wanigasekera (2000). For each strain, 35 mL culture volume of each of the salinity treatments, filled into ten replicates of 50 mL total volume FTs was used. For the bisexual strains (GSL and VC), a pair of copulating individuals was inoculated into each of the FT replicates while a single individual was used for the parthenogenetic strains (BLK and TUZ). The brood pouch in the females and hooked graspers in males aided to distinguish between the two sexes in the bisexual species (Van Stappen, 1996b). After the animals were inoculated, 1 mL (at a concentration of approximately 27.5 million cells mL^{-1}) of fresh *Tetraselmis suecica* algae were added as feed into each FT; the FT were placed in water baths in a temperature controlled room at 28 ^{o}C (Figure 3.3). Subsequent feeding was done *ad libitum* based on visual observation. FTs were monitored daily for mortality and reproduction. In the case of any mortality, the dead animal was replaced with a

living individual from the stock population of the same strain and salinity. For the sexual strains, dead males were replaced continually as long as the females were alive and a culture replicate was terminated once the female died. For the parthenogenetic strains, a culture replicate was terminated after the death of a reproductive female (Agh *et al.*, 2008). As for reproduction, all free swimming nauplii and cysts sighted in FTs were counted. This was achieved by pouring the entire content of the FT into a petri dish and offspring were pipetted under the binocular microscope. Parent animals were returned into fresh medium of the same salinity, fed and placed back into the water bath. Data collected were used to compute: i) female pre-reproductive period (days), ii) female reproductive period (days), iii) female post-reproductive period (days), iv) total lifespan of reproductive females (days), v) offspring as cysts, vi) offspring as nauplii, vii) offspring per day as cysts, viii) offspring per day as nauplii, ix) total offspring per female, x) total offspring per female per day, xi) number of broods per female, xii) offspring per brood, xiii) interbrood interval (days) and xiv) percent offspring encysted.

Figure 3.3: Falcon tubes filled with media of the different salinities, inoculated with the parthenogenetic individuals and bisexual couples, and placed in a water bath during the reproductive and life traits test

3.2.4 Statistical analysis

Analyses were performed using the SAS (SAS for Windows version 9.3) software. Data were arcsine transformed and the assumptions of normality and homoscedasticity were checked. The effects of three factors in Experiment 1 and 2 (salinity, strain and developmental stage at incubation) were included in a full model ANOVA analysis with their interactions. The dependent variable in both tests was the survival of the animals. Where interaction existed, one-way ANOVA between one main effect at each level of a second main effect was performed. For the reproductive and life traits, one-way ANOVA was employed to determine the effect of salinity and strain, respectively, on the reproductive parameters tested. One-way ANOVA was also used to test the differences between the cyst diameters of the strains used. For all the respective tests, where heteroscedasticity was observed, Welch's ANOVA was performed instead. Where ANOVA results indicated significance between the treatments, Bonferroni post-hoc test was performed to compare and identify differences between the means. P-values of less than 0.05 were considered

significant. As 100 % mortality was recorded at 0 g L^{-1} salinity for all the strains tested in Experiment 1 and 2, it was excluded from the analyses.

3.3. Results

3.3.1. Cyst diameter measurements of the four *Artemia* strains

Differences were observed between the cysts of the four *Artemia* strains. The mean cyst diameter of the different strains differed significantly (ANOVA, F = 162.01, P = 0.0001), with the VC strain having the smallest cyst diameter while TUZ strain had the largest (Table 3.2).

Table 3.2: Mean ± standard deviation (n = 100) of the cyst diameter (in µm) for the four *Artemia* strains. Different superscripts show differences in significance between values in the row

STRAIN			
TUZ	BLK	GSL	VC
275.2±10.3[a]	254.7±17.4[b]	242.6±11.4[c]	236.8±12.2[d]

TUZ = Tuz strain, BLK = Balikun strain, GSL = Great Salt Lake strain, VC = Vinh Chau strain

3.3.2. Effect of low salinity on the survival of instar I and II developmental stages of the different strains in axenic condition (Experiment 1)

Generally, a high survival of the different strains used was observed. A significant interaction among the three factors, i.e. salinity, strain and developmental stage, existed (F = 5.12, P = 0.0001). Salinity contributed most to the explanation of the result; hence, the data were sorted by salinity. When the interaction effect was removed and the data were further analysed using the one-way ANOVA, the results revealed that salinity had significant effect on survival with respect to the strain factor, whereas on the contrary, salinity had no significant effect on survival with respect to the instar developmental stage (Table 3.3). The post-hoc analysis showed that at 5 g L^{-1}, the BLK strain had significantly higher survival (96.7 ± 2.6 %; $P<0.05$) than the TUZ strain (71.7 ± 25.8 %), whereas the two sexual strains showed intermediate survival, not significantly different from either BLK or TUZ (Figure 3.4). At 32 g L^{-1}, it was the TUZ strain which had significantly higher survival (100.0 ± 0.0 %) than the BLK strain (94.2 ± 3.8 %), with again the sexual strains in-between. No significant difference ($P>0.05$) was observed between strains at 10 g L^{-1} (Figure 3.4) with minimum survival of 95.0 ± 3.2 % for the TUZ strain and highest survival 99.2 ± 0.8 % for the BLK strain. Differences in survival between the two instar stages were non-significant ($P > 0.05$) at each salinity (Figure 3.5).

Table 3.3: Results of ANOVA performed for the survival of four *Artemia* strains and two instar developmental stages in three salinities during the 48 h axenic test

Salinity (gL^{-1})	Strain		Instar developmental stage	
	F-ratio	P-value	F-ratio	P-value
5	4.04	0.021	0.64	0.433
10	1.73	0.193	3.36	0.081
32	3.62	0.031	0.000	1.00

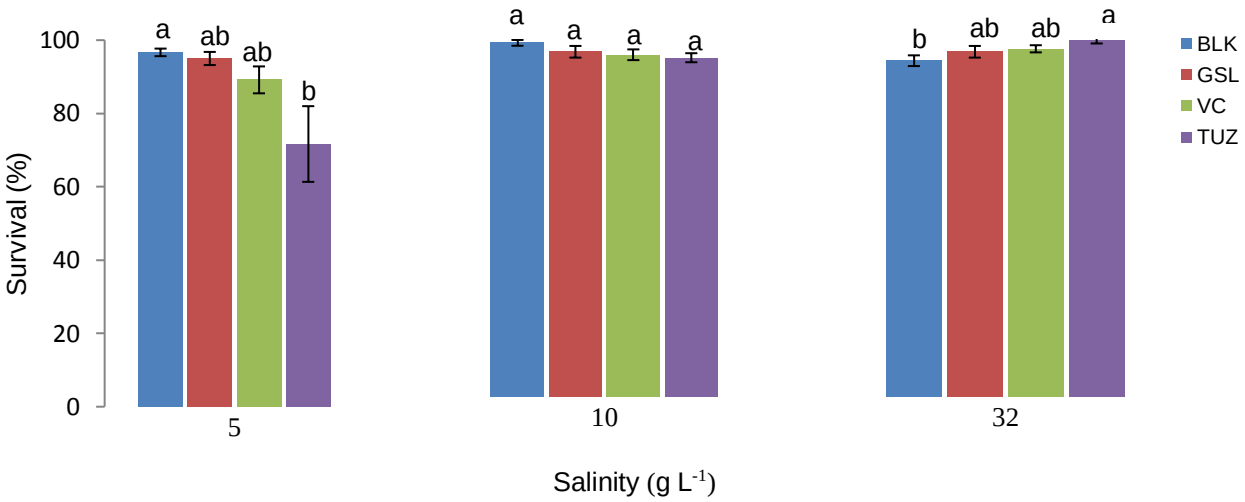

Figure 3.4: Mean (n=3) of the survival of four *Artemia* strains cultured at different low salinities during a 48 h axenic test. Different superscripts show significant difference ($P < 0.05$) between means per salinity. Error bars correspond to standard deviation

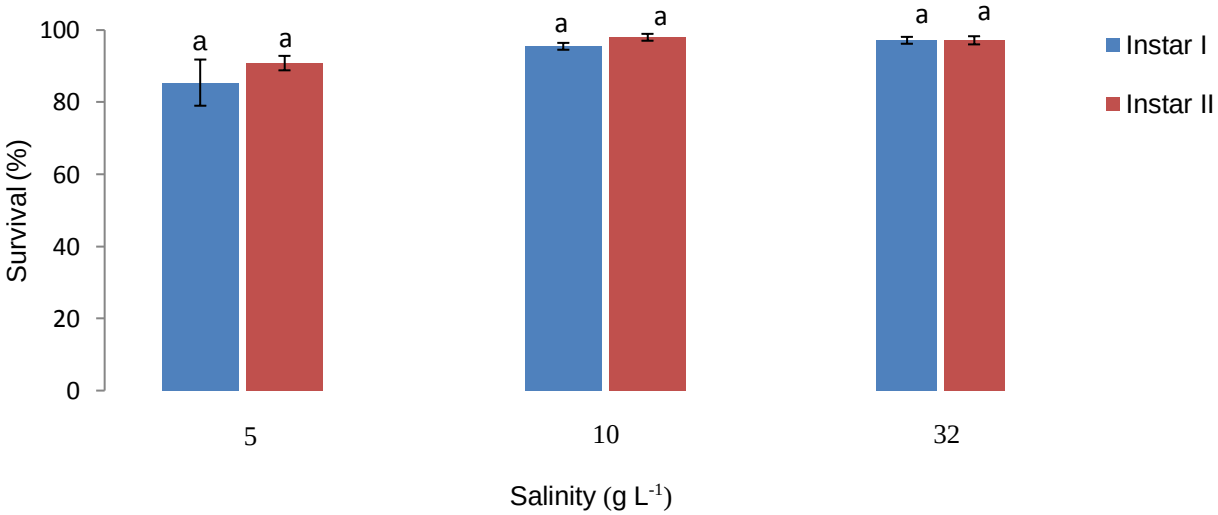

Figure 3.5: Mean (n=3) of the survival at instar I and II developmental stages of four *Artemia* strains cultured at different low salinities during a 48 h axenic test. Different superscripts show significant difference ($P < 0.05$) between means per salinity. Error bars correspond to standard deviation

3.3.3. Effect of low salinity on the survival of instar I and pre-adults in xenic conditions (Experiment 2)

Similar to the axenic test, high survival rate was also observed at most of the salinities tested during the xenic test, except at 5 g L^{-1}. Significant interaction also occurred between salinity, strain and incubation age (F = 13.53; P = 0.0001). Further, salinity had significant effect on survival with respect to both other factors: strain and incubation age (Table 3.4). Differences in survival were observed between strains at 20 and 32 g L^{-1} (P<0.05), but not at 5 and 10 g L^{-1} (Figure 3.6). Highest survival at 20 g L^{-1} (97.8 ± 2.1 %) and at 32 g L^{-1} (98.0 ± 2.0 %) was observed with the GSL strain, while the lowest survival (87.4 ± 10.4 %; 57.4 ± 35.9 % respectively), was observed with the BLK strain. At these two salinities GSL values were significantly higher (P < 0.05) than both TUZ and BLK values. Differences between both incubation ages were observed only at 5 g L^{-1} (Figure 3.7). The highest survival (93.6 ± 4.6 %) was observed when the animals were inoculated at 8 days at 20 g L^{-1}; however, this did not significantly differ from survival when inoculated at 0 days at the same salinity (P>0.05). This pattern was also observed at 10 and 32 g L^{-1} salinities where survival was also high.

Table 3.4: ANOVA results performed for survival of four *Artemia* strains and two incubation ages in four salinities during a nine day xenic test

Salinity (g L^{-1})	Strain		Inoculation age	
	F-ratio	P-value	F-ratio	P-value
5	2.67	0.054	26.96	0.0001
10	2.89	0.052	0.17	0.685
20	4.40	0.007	2.56	0.114
32	10.44	0.0001	0.23	0.636

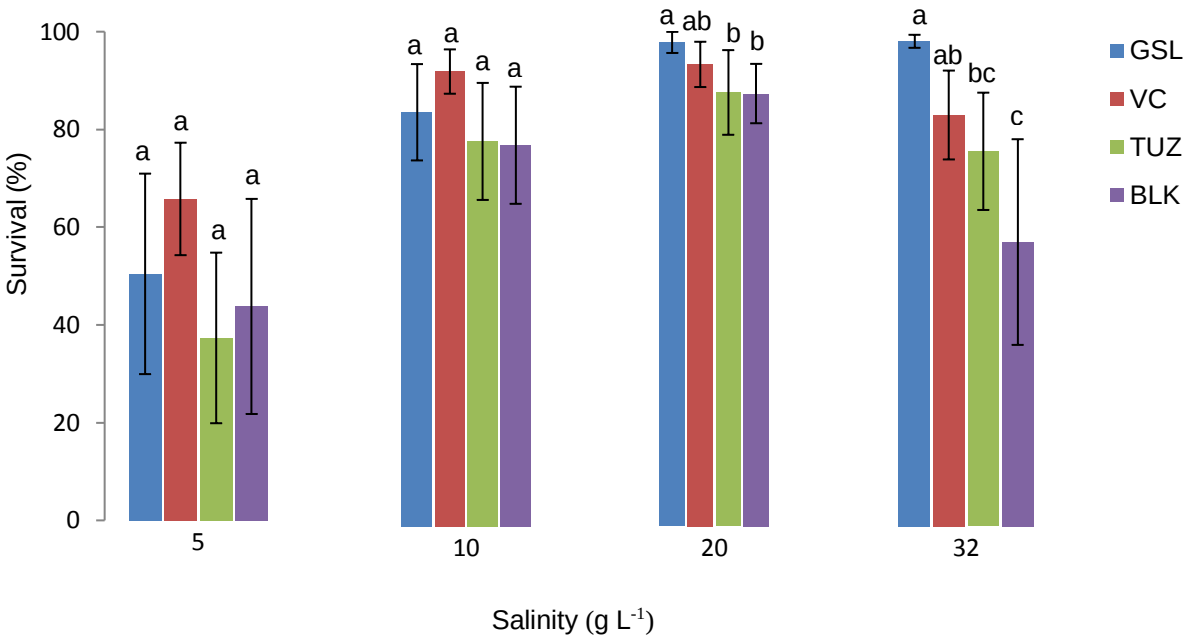

Figure 3.6: Mean (n=3) of the survival of four *Artemia* strains cultured at different low salinities during a nine day xenic test. Different superscripts show significant difference ($P < 0.05$) between means per salinity. Error bars correspond to standard deviation

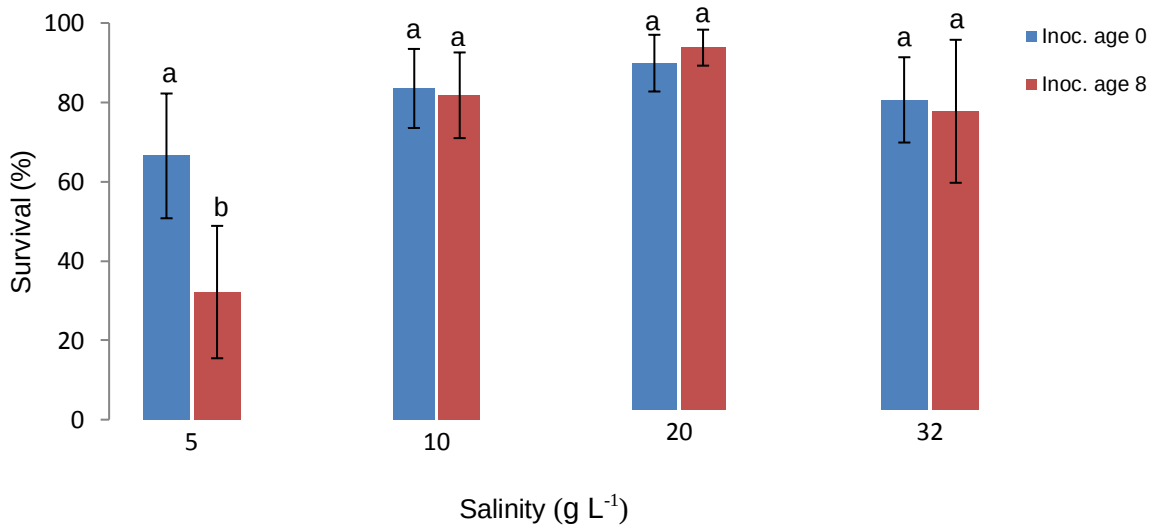

Figure 3.7: Mean (n=3) of the survival at inoculation age 0 and 8 days of four *Artemia* strains cultured at different low salinity during a fnine day xenic test. Different superscripts show significant difference ($P<0.05$) between means per salinity. Error bars correspond to standard deviation

3.3.4. Effect of low salinity on reproductive and life traits of the different *Artemia* strains (Experiment 3)

Due to total mortality of the animals at 0 g L^{-1} salinity during the xenic test (Experiment 2), only 5, 10, 20 and 32 g L^{-1} salinity were included in experiment 3. Mortality also persisted among the two parthenogenetic strains at 5 g L^{-1}, whereby no animal reproduced. Furthermore, although these two strains were observed to reproduce at 10, 20 and 32 g L^{-1}, neither had all animals reproducing

in any of the salinities. As for the sexual strains, reproduction occurred at all the salinities tested, including the lowest 5 g L^{-1}. Generally, the two sexual strains had a higher number of reproducing females than the parthenogenetic strains. The lowest percentage (20 and 30 %) of females that reproduced was recorded with the BLK and TUZ strains, respectively, at 10 g L^{-1} salinity. On the other hand, the highest percentage (100 %) of females that reproduced was observed with GSL at 20 and 32 g L^{-1} and with VC at 32 g L^{-1} salinity.

Both factors salinity and strain had a significant effect on a number of reproductive and life traits tested (Table 3.5). Out of the fourteen traits considered, salinity had a significant effect ($P<0.05$) on five (Table 3.6), whereas the strain factor had a significant effect ($P<0.05$) on 11 traits (Table 3.7). Salinity and strain each had a significant effect on three among the traits, i.e. female reproductive period, total lifespan of reproductive females and total offspring ($P<0.05$). Analysis for strain effect on female pre-reproductive period revealed that the GSL *A. franciscana* started reproduction significantly earlier (at 13.5 ± 3.9 days) compared to the parthenogenetic strains. Generally, more reproductive days were observed as salinity increased. A significantly longer reproductive period (4.4 days) was recorded at 20 and 32 g L^{-1} than at 5 and 10 g L^{-1} (resp. 0.7 and 1.2 days). BLK had the shortest period of reproduction (0.5 ± 1.5 days), while GSL reproduced for significantly more days (5.5 ± 4.6; $P < 0.05$).

The two parthenogenetic strains BLK and TUZ had a longer total lifespan (28.1 ± 11.6 and 23.8 ± 4.0 days, respectively) than the sexual ones, and in the case of BLK the difference with the sexuals was significant ($P<0.05$); no significant difference was found between the two parthenogenetic strains. Similarly, no significant difference was observed in the total lifespan of the two sexual strains VC (22.5 ± 3.1 days) and GSL (22.2 ± 4.5 days). With respect to the effect of salinity, significantly longer (27.9 ± 8.1) total lifespan was observed at 20 g L^{-1} than at 5 and 32 gL^{-1} (the difference with 10 g L^{-1} was not significant). GSL produced significantly more nauplii per female (200.5 ± 191.8) than the two parthenogenetic strains ($P<0.05$) (the difference with VC was not significant). The BLK strain had the lowest (12.5 ± 69.4) number of nauplii produced per female. The percentage offspring produced as cysts was the lowest in GSL and TUZ strains (26.1 ± 23.1 and 22.4 ± 35.6, respectively).

Table 3.5: ANOVA (P-values) for the effect of strain and salinity on reproductive and life traits of the different *Artemia* strains tested at the different experimental salinities

	Main effects	Female pre-reproductive period	Female reproductive period	Female post-reproductive period	Total lifespan of reproductive females	Offspring as cysts	Offspring as nauplii	Offspring per day as cysts	Offspring per day as nauplii	Total offspring per female	Total offspring per female per day	Number of broods per female	Offspring per brood	Interbrood interval	Percent of offspring encysted
P-value	Strain	0.0001	0.0001	>0.539	0.0040	0.0003	0.0007	0.0017	0.001	0.0001	>0.7939	0.0004	0.0001	>0.058	0.0001
	Salinity	>0.1451	0.0005	0.0015	0.0003	>0.3025	>0.0501	>0.5116	>0.4312	0.0151	>0.3113	>0.0615	>0.1799	0.0045	>0.9323

Table 3.6: Effect of different salinity levels on the reproductive and life traits of the different *Artemia* strains. Values represent mean ± standard deviation (n = 10). Different superscripts show significance between means in rows

Parameters	Salinity (g L^{-1})			
	5	10	20	32
Female reproductive period (days)	0.7±2.4^{b}	1.2±2.2^{b}	4.4±5.1^{a}	4.4±3.8^{a}
Female post-reproductive period (days)	1.0±1.2^{b}	3.5±3.4^{ab}	4.7±4.1^{a}	2.4±1.8^{b}
Total lifespan of reproductive females (days)	20.3±4.1^{b}	24.7±4.1^{ab}	27.9±8.1^{a}	23.7±3.3^{b}
Total offspring per female	56.6±101.6^{b}	110.3±95.9^{ab}	174.5±203.2^{ab}	214.4±203.2^{a}
Interbrood interval	0.3±1.1^{ab}	0.2±1.2^{b}	2.0±3.3^{ab}	2.7±2.9^{a}

Traits shown are for which significant effect could be found

Table 3.7: Effect of strain types on the reproductive and life traits of *Artemia*. Values represents mean ± standard deviation (n = 10). Different superscripts show significance between means in rows

Parameters	Strain			
	BLK	TUZ	VC	GSL
Female pre-reproductive period(days)	23.1±4.9^{a}	19.5±2.9^{a}	15.6±4.2^{b}	13.5±3.9^{b}
Female reproductive period (days)	0.5±1.5^{c}	1.2±3.3^{bc}	3.5±3.8^{b}	5.5±4.6^{a}
Total lifespan of reproductive female (days)	28.1±11.6^{a}	23.8±4.0^{ab}	22.5±3.1^{b}	22.2±4.5^{b}
Offspring as cysts	16.1±30.8^{b}	6.4±29.2^{b}	104.5±119.4^{a}	67.0±63.0^{ab}
Offspring as nauplii	12.5±69.4^{b}	41.4±52.8^{b}	115.5±198.2^{ab}	200.5±191.8^{a}
Offspring per day as cysts	26.2±29.4^{ab}	10.2±24.5^{b}	61.3±67.5^{a}	21.72±41.7^{b}
Offspring per day as nauplii	40.4±48.2^{ab}	66.3±38.5^{a}	23.1±28.6^{b}	62.5±53.1^{a}
Total offspring per female	36.6±68.9^{b}	58.2±42.5^{b}	188.9±179.9^{a}	272.1±207.0^{a}
Broods per female	1.0±0.5^{b}	1.0±0.4^{b}	1.7±1.0^{ab}	2.0±1.3^{a}
Offspring per brood	50.4±31.6^{b}	71.9±21.3^{b}	116.9±64.9^{a}	135.8±64.9^{a}
Percent offspring encysted	49.3±43.0^{ab}	22.4±35.6^{b}	66.1±41.8^{a}	26.1±23.1^{b}

TUZ = Tuz strain, BLK = Balikun strain, GSL = Great Salt Lake strain, VC = Vinh Chau strain. Traits shown are for which significant effect could be found

Table 3.8: Mean ± standard deviation (n = 10) values of reproductive and life traits of the four *Artemia* strains in the different salinities tested

Salinity	Strain	Parameters													
		Female pre-rep period	Female rep period	Female post-rep period	Lifespan repr females	Offspring as cysts	Offspring as nauplii	Offspring as nauplii day^{-1}	Offspring as cysts day^{-1}	Total offspring female^{-1}	Total offspring day^{-1}	Broods female^{-1}	Offspring brood^{-1}	Inter-brood interval	% offspring encysted
5	BLK														
	GSL	11.8±1.5	1.0±0.0	1.8±1.5	14.5±2.9	0.0±0.0	111.8±55.7	111.8±55.7	0.0±0.0	111.8±55.7	111.8±55.7	1.0±0.0	111.8±55.7	0.0±0.0	0.0±0.0
	TUZ														
	VC	16.5±4.1	2.9±2.8	1.1±1.1	20.5±3.0	102.5±96.8	43.0±96.2	10.1±23.8	53.8±43.6	145.5±120.2	63.9±37.5	1.5±0.4	88.0±42.0	1.5±2.3	84.7±34.8
10	BLK	16.0±0.0	1.0±0.0	0.0±0.0	17.0±.0.0	17.5±24.8	64.0±33.9	64.0±33.9	17.5±24.8	81.5±10.0	81.5 ±10.0	1.0±0.0	81.5±10.0	0.0±0.0	46.7±0.0
	GSL	17.9±3.7	3.7±3.0	3.4±3.4	25.7±4.2	72.0±57.5	180.4±69.4	72.6±53.1	42.8±60.8	252.4±96.3	115.4±112.2	1.9±1.0	154.4±90.0	1.5±1.5	24.6±19.8
	TUZ	18.0±0.0	1.0±0.0	2.0±1.0	21.0±1.0	6.7±5.8	88.0±17.3	88.0±17.3	6.7±5.8	94.7±23.9	94.7±23.9	1.0±0.0	94.7±23.1	0.0±0.0	6.2±5.4
	VC	17.9±3.4	1.3±0.7	5.4±3.5	24.5±3.0	73.9±50.2	35.6±71.3	18.7±30.0	73.9±50.2	109.5±43.4	92.6±23.5	1.1±0.4	96.8±21.4	0.3±0.7	72.3±45.2
20	BLK	24.8±5.5	1.8±1,8	4.4±5.2	38.6±15.8	33.2±32.0	84.6±111.5	50.1±78.1	33.2±32.0	117.8±109.8	83.3±92.6	1.4±0.6	70.6±47.4	2.0±2.7	45.0±51.2
	GSL	10.8±0.6	9.1±4.9	4.5±4.3	24.4±3.2	24.6±72.8	279.1±261.9	51.4±56.5	7.7±6.6	358.1±270.7	59.1±53.6	2.8±1.8	127.9±39.2	3.1±2.5	24.4±20.0
	TUZ	19.1±4.2	2.6±4.6	5.5±4.5	27.3±4.1	26.8±34.8	55.6±37.1	47.7±40.7	22.9±35.7	82.4±14.9	70.7±28.7	1.1±0.4	76.1±16.5	1.6±4.6	32.4±43.7
	VC	15.9±4.5	3.8±4.4	4.9±3.3	24.6±2.1	103.8±100.6	162.3±307.0	27.3±37.5	80.7±107.9	153.0±140.2	107.9±95.9	1.8±1.4	135.4±91.8	1.7±3.3	64.0±46.4
32	BLK	22.8±3.9	1.9±1.6	1.8±1.9	26.4±3.1	32.3±34.2	32.9±34.1	17.1±15.1	26.1±31.6	65.1±37.1	43.2±22.1	1.3±0.5	50.9±20.1	0.9±1.6	49.0±43.8
	GSL	13.2±3.9	6.5±3.6	3.1±1.7	22.7±1.9	95.5±52.3	226.4±198.5	40.0±37.9	33.7±48.5	321.9±202.0	73.7±77.6	2.1±1.0	155.2±71.7	4.3±2.9	37.2±25.8
	TUZ	19.4±1.6	2.8±2.4	2.9±1.7	25.0±3.3	16.1±28.8	104.5±65.7	64.2±39.3	4.1±6.2	120.6±58.4	68.3±36.5	1.4±0.5	83.4±25.1	1.8±2.4	15.3±32.5
	VC	12.5±3.2	6.2±4.3	2.1±2.0	20.8±2.1	139.9±183.4	211.1±175.7	30.6±21.2	39.5±48.1	338.0±242.9	70.1±42.1	2.2±1.0	143.2±66.8	3.6±3.1	47.6±37.7

3.4. Discussion

In nature, *Artemia* are inhabitants of varying saline environments. Different populations are adapted to certain specific salinity conditions, whereby some strains are found in environments with very high salinity (Gajardo & Beardmore, 2012). Nevertheless, when salinity tends towards high levels, *Artemia* becomes physiologically stressed (Van Stappen, 2008). This could affect normal body functions such as reproduction and may ultimately lead to mortality. On the other hand, it is known that this organism thrives well at sea water salinity (Van Stappen, 1996a). In the present study, it was hypothesized that low salinity levels could support good physiological functions in the animals which may result in high survival and ovoviviparity. Ovoviviparity in *Artemia* has always been considered to occur only when conditions are favourable and particularly at relatively lower salinity. Hence, different low salinity levels (0 - 32 g L^{-1}) were used to test the survival, reproductive and life traits of four *Artemia* strains (Great Salt Lake and Vinh Chau strains of *Artemia franciscana*; Tuz and Balikun strains of parthenogenetic *Artemia*). However, it has been reported that the instar I *Artemia* nauplius stage is more tolerant to high salinity shocks than later stages, including instar II (Vanhaecke and Sorgeloos, 1980a), but it has never been tested if this is also the case for low salinity shocks. Therefore, the performance of instar II and pre-adult *Artemia* was tested, after incubation in lower salinity, in comparison with instar I nauplii. Four different strains were used as it was assumed that low salinity tolerance might be strain-specific. Additionally, the reproductive and life traits of the strains used in the study were also assessed at the different low salinities. The aim of the study was to select the most suitable low salinity level and strain, as well as to obtain information on the most appropriate developmental stage for the purpose of inoculation during mass culture for continuous ovoviviparous nauplii production.

The selection of the strains for the test was based on their availability on the global cyst market and their stress tolerance. The Great Salt Lake is known to be a native North American strain of *Artemia franciscana* (Lavens & Sorgeloos, 2000). *Artemia franciscana* has an invasive ability; as a result, intentional and unintentional inoculations have been carried related with aquaculture activities (Ruiz, Amat & Navarro, 2008). The Vinh Chau strain of *Artemia franciscana,* whose origin is from the San Francisco Bay strain, is an example of such inoculations into Vietnam (Kappas, Abatzopoulos, Van Hoa, Sorgeloos, & Beardmore 2004). Investigations from multidisciplinary perspective, using reproductive characteristics, allozyme and mitochondrial DNA analyses, have shown an evident divergence between the SFB and VC strains, with the VC strain showing high tolerance to stress such as for example induced by temperature (Kappas, *et al.*, 2004). Cysts of *Artemia franciscana* from the coastal saltworks in San Francisco Bay, California, USA and from an inland biotope, the Great Salt Lake, Utah, USA have been exported worldwide since 1950 making them of high commercial relevance (Lavens & Sorgeloos, 2000). As for the two parthenogenetic strains, they are also harvested, but of more limited commercial relevance. They

were included because according to Browne and Wanigasekera (2000), parthenogenetic strains would be better adapted to extreme environmental conditions.

In a first experiment, the performance of instar I and instar II *Artemia* was compared in axenic conditions, which is also referred to as the gnotobiotic *Artemia* culture (GART) system (Baruah, Ranjan, Sorgeloos & Bossier, 2010). The GART system was developed for the study of host-microbe interactions and offers a unique way of eliminating the interference of all microbial communities that are naturally present in the rearing environment. Since the focus in the present study was to test the effect exclusively of salinity on the survival of the animals, the system was adopted in order to eliminate all such unknown microbial communities, which the test animals may feed on, hence increasing their tolerance and altering the effect of the salinity itself. Although the axenic test lasted for only 48 h, the absence of any form of bacteria in the culture media [apart from the autoclaved *Aeromonas harveyi* (LVS3) used as feed], allowed for a good insight on the effect of salinity and the other factors (i.e. strain and instar developmental stages) on survival. The observed high survival (≥90 %) at salinities from 5 – 32 g L^{-1}, suggests that this range of low salinity is highly supportive of the animals' performance within the culture period. The relatively lower survival (71.7 %) observed with the TUZ strain at 5 g L^{-1} could be indicating strain preference to different salinities. This was particularly observed with the two parthenogenetic strains. While it appears that the BLK strain may be better adapted to the lowest salinity tested, the TUZ strain on the other hand seems to prefer the highest salinity tested (Figure 3.4). The two bisexual strains on the other hand performed in a similar way at all the salinities tested. Salinity tolerance of *Artemia* is related to the activity of the Na, K-ATPase enzyme. With reference to the activity of this enzyme in the nauplii of *Artemia*, Lee and Watts (1994) showed increases of this enzyme in emerged embryos and during the first instar stage. Since the maintenance of balance of the enzyme in these developmental stages of the nauplii is by nature programmed to occur independent of the environmental salinity, it must be supportive of good performance such as survival in the animals. Although the Na, K-ATPase enzyme activity was not investigated in the present study, the non-significant difference observed between the survival rates of the two instar developmental stages of all the strains at the respective salinities tested (Figure 3.5) may suggest that the processes for the activity of the enzyme in instar II could be similar to that in instar I which is independent of the environmental salinity.

The first experiment was run in gnotobiotic conditions in order to exclude the effect of the microbial environment when testing for salinity tolerance of *Artemia.* In mass production of *Artemia*, however, the xenic approach is practiced, due to the increased size of the system and the longer culture duration compared to laboratory tests, which are mostly characterized by their small size and shorter duration. In practice, a continuous *Artemia* nauplii production system, which is supposed to run as an alternative to nauplii production through cyst hatching, would be operated

anyhow in xenic conditions. Such xenic systems are associated with microbial organisms of which some are pathogenic and could constitute huge challenges leading to mortality. Other microbial organisms on the other hand can have a beneficial effect, for example as they contribute to *Artemia* nutrition in the culture (Pablo & Jones, 1993; Toi, Boeckx, Sorgeloos, Bossier & Van Stappen, 2013). Maintaining favourable environmental conditions such as salinity levels which support efficient osmoregulatory processes within such systems could contribute to reduced physiological stress, and may result in increased survival rate. In the present xenic experiment, in which we tested for performance of the pre-adult stage at low salinity, in comparison to instar I nauplii, the high survival recorded at the salinity range from 10 to 32 g L^{-1} indicated that the animals performed well throughout this salinity range. But at salinity lower than 10 g L^{-1}, survival may be reduced significantly. Irrespective of the strain, the relatively consistent higher survival of the animals at 20 g L^{-1} suggests that this salinity was suitable for the different strains. The significantly ($P < 0.05$) higher survival rate (97.8 ± 2.1 %) (Figure 3.6) obtained with the GSL strain further indicated that the strain may be relatively more adapted to this level of low salinity. As in the test under axenic condition (Figure 3.4), a similar pattern also occurred in the xenic condition (Figure 3.6), whereby the BLK parthenogenetic strain appeared not so much affected by lower salinities as TUZ strain was affected. This supports the assertion that parthenogenetic strains of *Artemia* may be niche specialists (Browne & Wanigasekera, 2000).

Although *Artemia* adults and juveniles are known to be more tolerant to different environmental factors than younger stages (Wear & Haslett, 1986; Wear *et al.,* 1986), they are influenced by their ambient salinity (Holliday, Roye & Roer 1990). Comparing the effect of incubation into low salinity on freshly hatched *Artemia* nauplii and on 8 days-old pre-adults, the results indicated that the pre-adult *Artemia* could experience a shock when transferred from a higher to a relatively lower salinity medium. But this observation applies only to the lowest salinity tested (5 g L^{-1}) where the difference between the two age groups was significant. Since no significant difference was observed in survival between the two age groups at salinities in the range 10 – 32 g L^{-1}, it suggests that when using these latter salinities for mass culture purposes, it may not be needed to rear the animals to the pre-adult stage before they are incubated at lower salinity. Hence, it was concluded that using nauplii rather than pre-adults for the purpose of incubation at lower salinity would result in a more efficient use of time and other resources required in the culture of the animals.

For a successful mass culture, it is not only important that the animals survive at lower salinity, but also that they show a sufficiently high fecundity throughout their reproductive life, and that they reproduce mainly ovoviviparously. This was assessed in Experiment 3. Only the sexual strains completed their lifecycle in all the salinities where survival was recorded. On the contrary, the parthenogenetic strains could only reproduce at 10 g L^{-1} salinity and above. A similar observation was reported in a study with four sexual *Artemia* species and a parthenogenetic strain from

Margherita diSavoia (Italy), where the parthenogenetic strain reproduced only in two out of nine salinity and temperature combinations (Browne & Wanigasekera, 2000). The present observation again supports the description of the parthenogenetic strains as niche specialists. The higher number of reproductive females of the sexual strains in some of the salinities tested distinguishes them from the parthenogenetic strains in the present study. Noteworthy is that the GSL and VC strains had all (100 %) females reproducing at 20 and 32 g L^{-1}, respectively. For most of the traits assessed, the best performances were recorded at 20 g L^{-1} salinity. Since the overall objective is to achieve ovoviviparity during mass culture, only reproductive traits which are more relevant for this purpose are discussed here.

The importance of longer reproductive periods cannot be over-emphasized. More reproductive days observed in the GSL strain and at 20 g L^{-1} salinity may have contributed to the higher number of total offspring recorded for this strain. However, the number of days ranging 4.2 – 14 recorded in the present study falls short of the 23.2 days recorded by Soniraj (2004) with the Tuticorin strain of *Artemia franciscana* at 20 g L^{-1} salinity. Agh *et al.* (2008) reported 28.4, 18.0 and 20.8 reproductive days for a parthenogenetic strain from Urmia Lake, Iran, a parthenogenetic strain from lagoons around Urmia Lake and the sexual *Artemia urmiana*, respectively, but at 75 gL^{-1} salinity. Comparing results in the present study with that of Soniraj (2004) considering that the Tuticorin strain belongs to the same species with the GSL strain, and that it was also tested at 20 g L^{-1}, the difference observed may be due to differences in adaptation of the animals to specific local environmental conditions, or to differences in other experimental conditions.

Generally, the ovoviviparity observed in all salinities, at which the parent animals survived, also suggests a physiological response by the animals in relation to the salinity tested. It was clear that more nauplii (200.5 ± 191.8) were produced by the GSL strain of *Artemia franciscana*. This is high compared to the 126.7 total nauplii production per female throughout her lifetime, reported by Soniraj (2004) at 20 g L^{-1} salinity using the Tuticorin strain. However, the parthenogenetic strain used in their study produced a far higher (1,041.67) number of nauplii at the same salinity. With respect to the total offspring produced per female, the GSL strain showed a higher fecundity, though not significantly different, than the VC strain. The significantly higher total number of nauplii produced by the GSL strain, as compared with the other strains that were used, coupled with the high number of nauplii (62.5) per day indicated that the GSL strain is a better candidate for use in the mass culture.

Finally, the cyst diameter of the different *Artemia* strains used in this study was measured in order to obtain biometric information. It is known that there is a strong correlation between *Artemia* strains and cyst diameter (Vanhaecke and Sorgeloos, 1980b) as well as a positive correlation between cyst diameter and length of instar I nauplii (Van Stappen, 1996b). Although no biometrical

characteristics of nauplii were measured in the present study, the nauplius size (either oviparous or ovoviviparous) of the different strains was expected to vary in relation to their respective cyst diameters measured. This was also considered as an important criterion in the selection of the strain as the nutritional effectiveness of a food organism is primarily determined by its ingestibility and as a consequence by its size and form (Merchie, 1996). Among the strains used in the present study, only the GSL strain was part of the 17 strains from 14 countries whose biometrics were studied by Vanhaecke & Sorgeloos (1980b). The measurement (242.61 µm) of the GSL strain cyst diameter in this study also comes close to the values reported by these authors, which showed the hydrated cyst diameter for two GSL batches to be 252.5 µm and 244.2 µm. The cyst diameter of the GSL strain measured in the present study was found to be bigger than that of the VC strain, but it was significantly smaller than the cysts of the parthenogenetic strains used.

3.5. Conclusion

Based on results of the axenic test, it could be concluded that low salinity within the range tested, with exception of 0 g L^{-1} culture medium, supported high survival within 48 h of incubation for any of the two instar stages tested. The nine day xenic test also indicated that a high level of survival can be expected at a salinity as low as 10 g L^{-1}, but higher survival response at 20 g L^{-1} allows for the conclusion that this low salinity level is the best relative to the others tested. With regard to the appropriate age for inoculation into low salinity, since no significant difference existed between the two inoculation ages tested at a salinity range of 10 – 32 g L^{-1}, it was concluded that to save resources such as time, energy and funds, it will be more beneficial to inoculate *Artemia* for low salinity mass culture at the nauplius stage. Considering the high survival recorded at 20 g L^{-1} salinity particularly with the GSL strain, coupled with better performance in terms of the reproductive and lifetime traits measured with this strain, it was concluded that this salinity and strain are the most suitable for application in the mass culture and for subsequent experimental work.

Chapter 4

Performance of the Great Salt Lake strain *Artemia franciscana* fed with low cost agricultural materials as sole diets

CHAPTER 4

PERFORMANCE OF THE GREAT SALT LAKE STRAIN *ARTEMIA FRANCISCANA* FED WITH LOW COST AGRICULTURAL MATERIALS AS SOLE DIETS

Abstract

Due to the non-selective feeding habit of the brine shrimp *Artemia*, different feed types including the relatively affordable/universally available agricultural based materials are used for culture purposes. However, knowledge on the use of agricultural materials for culturing of a reproductive biomass is scarce. *Artemia* is known for bio-converting feed with poor nutritional quality into high quality animal protein. This study aimed to assess the effect of five locally sourced agricultural based materials consisting of pellets (i.e. by-products) of three crops (canola, oat and barley), grains of two crops (whole wheat grain and whole barley grain) as sole diet on growth, survival and their feasibility for rearing of reproductive biomass of the Great Salt Lake (GSL) *Artemia franciscana* at low (20 g L^{-1}) salinity. Two separate tests were conducted which involved firstly a 9-days small scale (using 500 mL glass bottles) feeding experiment in order to screen the feed materials. This first test was followed by up-scaling and mass culture of the *Artemia* from the nauplii stage and through their reproductive stage (43 days), using the feed materials selected from the first experiment. For the feed screening and up-scaled biomass tests, highest survival (81.3 ± 9.9 and 79.7 ± 4.6 %, respectively) and growth in length (5.9 ± 0.3 and 6.1 ± 0.8 mm, respectively) were observed in the treatment fed a stock suspension prepared from barley pellets (ANOVA; $P<0.05$). Similarly, in the biomass culture, descriptive statistics indicated higher individual weight gain (87.5 ± 7.5 µg), higher efficiency in feed conversion (1.2 ± 0.1), protein efficiency (5.7 ± 0.0) and growth rate (17.7 ± 0.4 % day^{-1}) in the group fed with the barley pellet stock. Nauplii reproduction started on day 21 after inoculation whereby daily harvest was maintained for 22 days. Within this period, an estimated total of approximately 135,000, 119,000 and 1,700 nauplii were harvested from the biomass fed stock suspensions prepared with barley pellet, oat pellet and whole barley grain, respectively. Despite low levels of nutrients of the different feed materials, the adults fed with the test feed and their ovoviviparous nauplii were found to contain appreciable levels of protein (33.34 – 35.25 and 36.13 – 41.31, respectively), essential and non-essential amino acids as well as saturated, mono-unsaturated and poly-unsaturated fatty acids in their tissues. The study revealed that feed materials such as the barley pellet could be used for the culture of actively reproducing biomass of the GSL strain of *Artemia franciscana*. Manipulating other agricultural based feed with higher nutritional value could, however, yield better results in terms of increased fecundity.

4.1. Introduction

Artemia are non-selective, obligate particle-filter feeders (Pravasoli & Shiraishi, 1959; Barker-Jorgensen, 1966; Dobbeleir *et al.,* 1980; Dhont & Lavens, 1996). In their natural habitat they are known to feed on live micro-algae as well as on bacteria, particulate matter and detritus. For laboratory culture purposes which are usually conducted at small scale, e.g. in falcon tubes, test vials or small aquaria, live microalgae are also regarded as the best for *Artemia* (Dobbeleir *et al*., 1980; Dhont & Lavens, 1996). Also, for short indoor biomass culturing of *Artemia* (e.g. to study reproductive behaviour or strain characteristics), or as an intermediate production scale for later outdoor up-scaling, again microalgae are often a good choice. Conversely, for mass indoor culturing of *Artemia* over a prolonged period, in which the animals are supposed to reproduce over several generations, the use of algae becomes much too costly and an alternative food which is more user-friendly is needed. Due to limitations owing to insufficient supply, labour as well as associated costs, the use of algae has been considered economically non-realistic when it is acquired or produced solely for the purpose of mass culturing of *Artemia* biomass (Lavens, De Meulemeester & Sorgeloos, 1987; Dhont & Lavens, 1996; Naegel, 1999; Hoa, Anh, Ngan, Toi & Le, 2007). Additionally, several species of algae are equally not void of other limiting factors common with many food materials used in animal production. Species such as *Chlorella, Coccochloris* and Dinoflagellates respectively have been identified to have thick cell walls, or secreting gelatinous or toxic substances (Dhont & Lavens, 1996). Varying biochemical composition of different algal species has been reported, which consequently reflects on *Artemia* biomass and their nauplii fed with these algae (Merchie, 1996). These factors have ensued investigations into alternatives which are less expensive yet could serve as good feed source for *Artemia*.

Several alternative feed sources have already been tested for *Artemia* culture such as dried algae (*Chlorella, Scenedesmus, Spirulina),* baker's yeast and various inert products such as fish meal, egg yolk and homogenized liver (Dobbeleir *et al.,* 1980). Others which have also been successfully used include coconut meal and sugarcane molasses (Persoone, 1978 cited in Dobbeleir *et al*., 1980; Talloen, 1978). These feed sources have also been shown to have limitations. Dhont and Lavens (1996) asserted that although algae in their dried form yielded satisfactory results, they have also been associated with high costs as well as high fractions of water soluble components which the organisms cannot ingest, leading to deterioration of the water quality within the culture medium. Yeast on the other hand has a small cell diameter with fairly complete nutritional composition, and a rigid cell wall which prevents leakages of water soluble components (Dhont and Lavens, 1996). However, digestibility could constitute a problem due to the thick yeast cell wall and therefore these cells need a certain treatment and/or supplementation for optimal culture results (Coutteau, Bendonck, Lavens & Sorgeloos, 1992).

Also, agricultural crops constituting mainly grains and their by-products have been successfully utilized for rearing of *Artemia.* Although certain practical limitations such as low nutritional composition, particle size, poor storage ability (in the case of wet feed) have also been associated with these agricultural materials, they are considered affordable and universally available (Sorgeloos *et al*., 1980; Dhont & Lavens, 1996). The by-products of ingredients/materials used in human and livestock food industries are particularly more affordable. When selecting these agricultural materials as *Artemia* feed, their generally poor nutrient composition has been regarded as less critical (Dhont & Lavens, 1996). Attributes which are considered as important criteria include cost, availability, palatability, solubility, digestibility, buoyancy, particle size and shelf life (Dhont & Lavens, 1996). This assessment is on the basis that certain bacteria and protozoans within the culture medium use the feed as substrate to biosynthesize essential nutrients, which compensates for the deficiency in the supplied feed. Also, it has been shown that *Artemia* are very efficient nutrient converters when they were fed on rice bran (Sorgeloos *et al*., 1980). High density culture of biomass with subsequent ovoviviparous nauplii production has been achieved using these less expensive agricultural materials (Lavens & Sorgeloos 1987).

In view of the overall goal of searching for alternative ways for the supply of *Artemia* as live food for hatcheries in areas which currently depend on the importation of cysts, this study aimed at a following step towards the development of a system for the continuous and affordable indoors mass production of ovoviviparously produced *Artemia* nauplii. In this study, we wanted to assess if the ovoviviparous mode of *Artemia* reproduction is biologically and technically feasible with biomass reared with agro-based materials as feed. Hence, the effect of some locally available agro-based materials as sole diets was tested on the performance of the Great Salt Lake strain of*Artemia franciscana.*

4.2. Materials and methods

4.2.1. Protocol review, ethics clearance and experimental permits

All protocols employed in this study were ethically reviewed and approved (Protocol #: SUACUD15-00002) by the Research Ethics Committee: Animal Care and Use via committee review procedures of the Stellenbosch University of South Africa. Also, a permit (No.1507598) for scientific investigation and practical experiments was obtained from the Department of Agriculture, Forestry and Fisheries of the Republic of South Africa in conjunction with the Western Cape Nature Conservation Board in terms of section 83 of the Marine Living Resources Act, 1998 (Act No.18 of 1998) and the Western Cape Nature Conservation Laws Amendments Act no 3 of 2000 (Incorporating nature conservation ordinance 19 of 1974).

4.2.2. Experimental site, design, feed materials, *Artemia* strain and culture conditions used

The study was conducted at the aquaculture facility of the Welgevallen experimental farm, Stellenbosch University, South Africa. Two separate experiments were conducted. In the first experiment, screening of feed materials was done in order to select the most suitable material(s) for use during mass culture. The second experiment involved up-scaling and mass culture of the *Artemia* from the nauplii stage onwards and through their reproductive stage, using the feed material(s) selected from the first experiment.

A total of five locally sourced agricultural based materials consisting of pellets (by-products) of three crops [canola (CP), oat (OP) and barley (BP)], and grains of two crops [whole wheat grain (WWG) and whole barley grain (WBG)] (Figure 4.1) were used as feed, along with a commercial *Artemia* feed (VF), produced by Can Tho University (CTU), Vietnam, for *Artemia* production in ponds, which served as a control. The locally sourced agricultural materials were micronized following the mechanical dry grinding procedure (Dobbeleir, *et al*., 1980) using a grinding machine (Figure 4.2). In order to ensure that the particles of the respective materials were within the optimal size range (2 - 50 µm), enabling uptake by *Artemia*, the ground fine powder was further processed by manual dry sieving through a net of 50 µm mesh size. The sifted materials were packed in air-tight, moisture-free plastic bags and refrigerated at 4 ˚C until needed.

Before the commencement of the first experiment and in order to confirm that the *Artemia* were able to ingest the particles of the materials, stock suspensions of the respective materials were prepared and tested using 20 *Artemia* per treatment, hatched from the Great Salt Lake (GSL) strain of *Artemia franciscana* (selected as best on the basis of performance: see Chapter 3). The guts of the *Artemia* were microscopically examined over nine days whereby compacted food material was observed within their guts and faecal matter within samples of the culture water.

Culture of *Artemia* was done at 20 g L^{-1} salinity in both experiments. Within the salinity range 5 – 32 g L^{-1}, 20 g L^{-1} had proven in our initial tests (see Chapter 3) to result in similar *Artemia* survival and reproduction as in standard seawater of 32 g L^{-1}. This salinity was achieved by mixing tap water from Stellenbosch municipal supply with natural 32 g L^{-1} seawater, collected from the research facility of the Department of Agriculture, Forestry and Fisheries, Sea point, Cape Town, South Africa. The desired salinity was checked using a refractometer (Hanna Instrument: HI 96822). Temperature of approximately 28 ˚C was used for both experiments (Dhont & Lavens, 1996). Animals were exposed to 12 h:12 h light and dark regimes. The xenic approach was used in all the experiments.

Figure 4.1: Raw (upper row) and micronized (lower row) forms of the different locally sourced agricultural based materials used as feed in the experiments. 1(a and b) = whole barley grain; 2(a and b) = canola pellet; 3(a and b) = oat pellet; 4(a and b) = barley pellet; 5(a and b) = whole wheat grain

4.2.3. Biochemical analyses

4.2.3.1. Proximate analysis

All the feed materials (except the CTU feed) were subjected to biochemical analyses on dry weight basis. Proximate composition, including analysis of crude protein, moisture, ash and crude fibre, was determined according to AOAC (2002) methods. For the crude protein, the Dumas method using LECO FP 528 for the quantitative determination of nitrogen in samples was used (AOAC, 2002) and calculation was performed using the conversion factor of 6.25. Moisture was determined by drying of samples at 100 ˚C for 24 h. Crude fibre was obtained gravimetrically. Crude lipid was analyzed by acid hydrolysis (AOAC, 2000). Carbohydrate was determined by difference (FAO, 2003). Gross energy was obtained using the CP00 bomb calorimetric method. Duplicate samples were used for each of the feed materials and parameters analyzed.

4.2.3.2. Amino acids analysis

Amino acids were measured in solutions after hydrolysis of proteins using standard 6M HCl acid digestion (AOAC, 2003). This analysis involves the standard Waters method using an Ultra C_{18} 2.1 x 100 mm x 1.7 µm column and derivatizing agents [6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC)]. After dilution and derivatization, amino acid separation and detection was performed using a Waters Acquity Ultra Performance Liquid Chromatography (UPLC) fitted with a photodiode array (PDA) detector. Single representative samples were analyzed. As for the *Artemia* biomass and ovoviviparous nauplii, each representative sample was a combination of sub-samples from the replicate tanks per treatment. Furthermore, samples for the ovoviviparous nauplii were constituted of several harvests.

4.2.3.3. Fatty acids analysis

The long chain fatty acids methyl ester (FAME) method for extracting long chain fatty acids (Folch, Lees and Sloane-Stanley, 1957; Pomeranz and Meloan, 1971; Heimann, 1980) was used to analyze fatty acids. A ThermoFinnigan Focus GC (Thermo Electron S.p.A., StradaRivoltana, 20090 Rodana, Milan, Italy) instrument was used with BPX70, 60 m x 0.25 mmID, 0.25 µm column (SGE International Pty Ltd, 7 Argent Place, Ringwood, Victoria 3134, Australia). The FAMEs were identified by comparison of the retention times to those of a standard FAME mixture (Supelco 37 Component FAME mix, C4 – C24, Cat no. 47885-U, 595 North Harrison Rd, Bellefonte, PA, 16823-0048, USA); 0.1 g sample was weighed off into a Kimax tube before 1 mL hexane and 50 µL C17:0 (internal standard, 10 mg mL^{-1}) were added. Thereafter, 2.5 mL H_2SO_4 in MeOH was added before the cap was screwed, vortexed and incubated at 80 °C for 1 h in an oven. Then tubes were allowed to cool down before 2.5 mL of 20 % NaCl solution (w/v) was added, vortexed, and tubes were allowed to stand for layers to separate. Once the top layer was clear, it was transferred to a GC vial (with insert) using a glass Pasteur pipet and analysed. Also, single representative samples (as described for the amino acids) were analyzed.

4.2.4. Screening of feed materials on a laboratory-scale (Experiment 1)

All the five feed materials together with the control feed were tested for their effect on survival and growth of *Artemia*. The micronized powder of the different feed materials was used in mixing of a stock suspension daily. To mix the suspension, a known weight of each of the different micronized feed materials was blended in a known volume of water of the same salinity as the culture medium (Figure 4.2). To determine the needed daily weight of the micronized feed materials, the feeding protocol of Coutteau *et al.* (1992) was adapted (Table 4.1) and calculation (formula 1) was performed based on 5,000 nauplii L^{-1} stocking density for the mass culture. The required volume of water was determined using formula 2.

Nauplii were hatched from the cysts of the GSL strain using standard procedures (Van Stappen, 1996a). Transparent glass bottles of 500 mL total volume filled with 300 mL culture water were used to inoculate the freshly hatched nauplii at 50 individuals per bottle. Three replicates per treatment were used in a randomized design and the experiment lasted for 9 days. The bottles containing the animals were placed in a water bath with temperature maintained at approximately 28 °C using 100 W submersible automatic aquarium water heaters. Continuous aeration was supplied from an air compressor connected to a series of 5 mm diameter rubber tubing placed into each of the bottles. Feeding commenced a few hours later. A total daily dose of 8 mL stock suspension per bottle was administered at 4 mL every 12^{th} hour feeding period (Dobbeleir *et al*., 1980, Evjemo & Olsen, 1999). The transparent glass bottles allowed easy visual inspection for behaviour of the animals and food availability.

Culture water was changed every third day. This was done by gently pouring the entire content of the culture bottle through a 100 µm mesh filter; trapped animals were immediately washed into a petri dish containing freshly prepared medium of the same salinity. Culture bottles and filter were carefully checked and properly rinsed to ensure no animal was lost. Animals were then carefully pipetted back into the rinsed bottles containing freshly diluted medium of the same salinity. The bottles were returned to the water bath and the animals were fed. Changing of the culture water ensured the maintenance of the desired salinity and reduced deteriorating water quality.

Survival and growth in length were determined at the end of the experiment. For survival, all the animals were counted under the binocular microscope, while final length of five randomly sampled individuals (10 % of initial density) per replicate was measured. Measurements were taken from the tip of the head to the end of the telson (Coutteau *et al*., 1992), also under a binocular microscope fitted with measuring ruler.

Figure 4.2: Grinding machine used for micronizing the different agricultural materials (left picture); a session during blending of micronized feed material into stock suspension used in feeding *Artemia* (right picture)

Table 4.1: Protocol for calculating the daily weight of micronized feed materials used to prepare the stock suspension

Day(s)	Coefficient
1	0.0154
2,3,4	0.0305
5,6	0.0462
7	0.0610
8	0.0776
9	0.1256

Coefficients are values that have been adapted from Coutteau *et al.* (1992) and are successfully being used in determining daily weight of feed material in the biomass production of *Artemia* in ponds (Hoa *et al*, 2007)

$$\text{Weight (g) of feed material day}^{-1} = \frac{\text{Biomass stocking density x Coefficient}}{1000} \quad (1)$$

Volume (mL) of water required
for mixing stock suspension = Calculated weight (g) of feed material day^{-1} x 100 (2)

4.2.5. Mass culture of *Artemia* on a pilot-scale (Experiment 2)

4.2.5.1. Culture set-up

From Experiment 1, feed materials which yielded up to 50 % survival were selected and used as treatments (feed) in order to test the performance of a high density *Artemia* biomass culture system during a more prolonged (six weeks) culture period. A water recirculation system (WRS) was constructed and used for rearing of the animals (Figure 4.3). The system consisted of six 21 L rearing tanks each measuring 37 cm x 26 cm x 25 cm. These were mounted on a metal platform directly above a 360 L water holding tank with a dimension of 60 cm x 185 cm x 39 cm, and partitioned into three separate compartments which served for particle sedimentation, biofilter and reservoir respectively. The rearing tanks were filled to 15 L culture volume, hence the total volume in the entire system was approximately 450 L. Water from the reservoir compartment was pumped into circulation (first to the rearing tanks) using a submersible water pump, and from the rearing tanks, it drained by gravity to the stock tank, then gradually flowing over a partition into the biofilter which was stocked with sponge-like materials to increase surface area for bacterial growth, upon which a commercial suspension of nitrifying bacteria was added. In this system, there was no filtering component but the spongy material trapped particles and was washed periodically. The water inflow into each rearing tank was regulated in the range of 0.4 - 2 Lmin^{-1}. The minimum flow rate was maintained during feeding and it was increased to the maximum when the culture water was clear, indicating low density of feed particles within the water column. This variable flow was adopted to allow longer retention time (about 10 min) of feed particles within the culture tanks at each feeding period and subsequently for the fast removal of faecal particles and possibly toxic dissolved nitrogenous compounds from the tanks.

Cysts were incubated and hatched using the same procedures as above, and the culture tanks were stocked with instar I nauplii at a density of 5,000 individuals L^{-1} (Dhont and Lavens, 1996) based on nauplii counting, using six samples of 50 µL of nauplii stock suspension. This stocking density was also used to calculate the needed volume from the stock suspension. Two tanks were assigned per treatment and distributed randomly.

Water level in the system was adjusted if needed by adding tap water to compensate for evaporation. Temperature was regulated by using a heat exchanger with a thermostatically controlled switch and copper tubing placed in the reservoir for heating the large volume of water. Additional 100 W submersible automatic aquarium water heaters also set at the same temperature were placed into each rearing tank. Following recommendations (Lavens and Sorgeloos, 1987) for

constant high oxygen level and optimal culture water exchange, a constant supply of oxygen within the tanks was maintained while 25 % of the culture water was exchanged weekly.

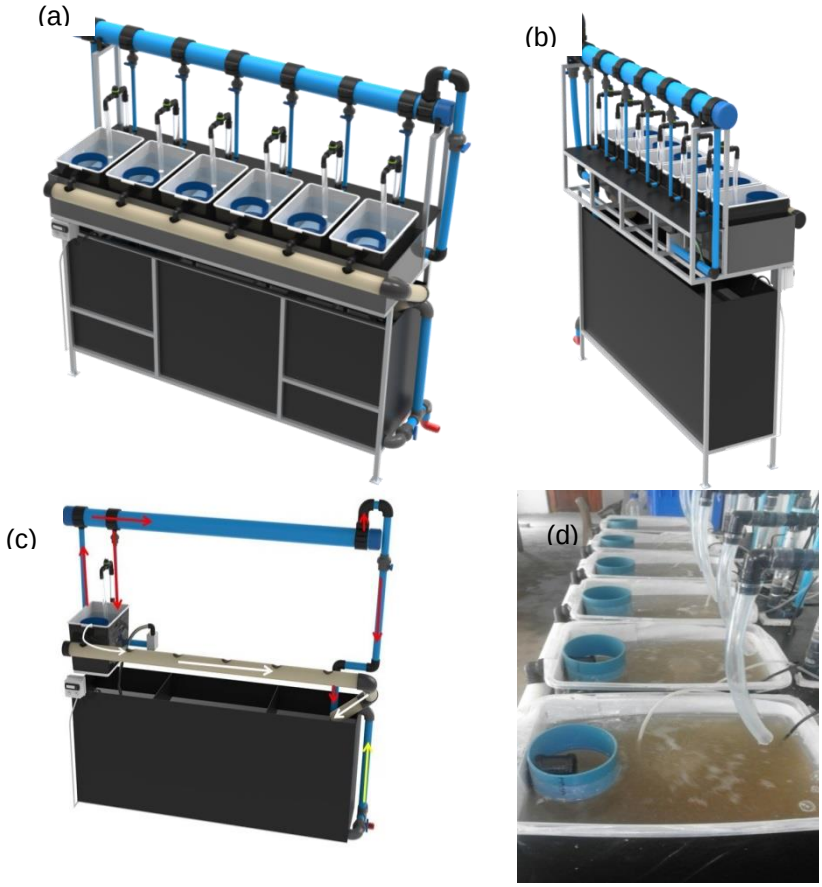

Figure 4.3: Water recirculation system used for testing the performance of the high density *Artemia* biomass. (a, b, c) = 3-D prototype model showing the front and back views, and directional water flow, respectively; (d) = a top view of the actual rearing tanks during culture operation

4.2.5.2. Feeding of biomass

Feeding of the biomass during the first nine days of culture was done twice a day and also according to the protocol in Table 4.1. As from the 10th day onwards, it was based on a ratio of approximately 10 % dry weight feed to live weight of biomass (Lavens & Sorgeloos, 1987). Live weight of biomass was obtained by pipetting the animals from the respective tanks and filtering them through a sieve. Thereafter, the animals on the sieve were rinsed with tap water to remove particles. The sieve was dabbed with paper towel and the animals were transferred into a clean pre-weighed aluminium foil cup. Foil cups containing animals were placed directly on a digital analytical balance and weight taken to the nearest 0.1 mg. Live weight was calculated according to the formula:

$$\text{Live weight biomass } (gL^{-1}) = \frac{\text{Total weight- Weight of empty foil cup}}{\text{Volume of sampled culture water}}$$

The different stock suspensions were prepared daily according to the same procedures as described in experiment 1 and refrigerated at 4˚C until needed.

4.2.5.3. Tank cleaning, water exchange and measurements of physico-chemical parameters

In order to maintain good water quality within the culture system, all tanks were cleaned every other day as from the fifth day after the initial stocking of animals. This was done by siphoning all animals in each tank into a separate tank containing water of the same culture salinity with a source of aeration, which was afterwards returned when the tanks were cleaned. Weekly 25 % of the total water volume in the system was exchanged. Water quality parameters were analysed using standard procedures as described in the manuals of the analytical instruments used. Dissolved oxygen (DO), temperature and pH were checked daily using the IP 67 model combo multi-function waterproof meter. Ammonia (NH_3), nitrite (NO_2^-), and nitrate (NO_3^-) was checked weekly using HACH's DR/850 model colorimeter following salicylate, diazotization, and cadmium reduction methods, respectively.

4.2.6. *Artemia* parameters measured

4.2.6.1. Survival

Survival was determined on days 1, 2, 3, 5, 7, 9, 14 and subsequently weekly. Triplicate samples from each tank were collected into 50 mL falcon tubes using a 25 mL pipette. Samples were then transferred into separate petri dishes and several drops of lugol solution were added and allowed for some time to act until all animals were fixed and deeply stained. Total animals in each sample were determined by placing the petri dish on a grid and counting using a hand magnifier (x10 magnification). The number of animals L^{-1} was determined and percent survival calculated.

4.2.6.2. Individual wet and dry weight of animals

Individual wet and dry weights of animals were obtained following methods described by Ownagh, Agh and Noori (2015). Data for the initial weight were obtained using duplicate samples each consisting of 500 freshly hatched instar I nauplii while the weight of pre-adults on day nine of the experiment was considered as final weight. Also, 500 individual animals (pre-adults) were collected by pipetting and filtering them through nets of appropriate mesh sizes. For the freshly hatched nauplii and the pre-adults respectively, 125 and 300 µm were used. The animals were rinsed with tap water to remove particles and were dabbed with paper towel before transferring them into a clean pre-weighed aluminium foil cup. Wet weight was obtained by directly placing the foil cups containing animals on a digital analytical balance and readings were taking to the nearest 0.1 mg. Dry weight was obtained by transferring the foil cups containing the animals into an oven at 60 ˚C for 24 h. Moisture-free samples were re-weighed after cooling in a desiccator for 30 min. Both the wet and dry weight readings were divided by the number of the animals sampled. Data obtained

were used to calculate the growth parameters: specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) (Table 4.2).

4.2.6.3. Biomass production

Biomass production was also measured at day nine. From each replicate, 2 L of culture water was siphoned over a 300 µm pre-weighed mesh. Samples were rinsed with tap water, dipped with a paper towel and wet weight biomass was determined (Table 4.2).

4.2.6.4. Length

Data for length were obtained on days 1, 2, 3, 5, 7 and 9. Freshly hatched instar I nauplii were used to measure initial length whereas sampled animals, used for estimation of the survival, were equally used for measuring of the length. Measurements were taken using 20 animals per replicate following the same procedure as described in Experiment 1 above.

4.2.6.5. Sex ratio

Sex ratio (%) was estimated following Coutteau *et al.* (1992). Estimation was done based on the percentage of total number of animals in tanks. Triplicate samples from each treatment replicate collected for survival estimation on day 21 were used. At this growth stage, male animals were identified by their hooked graspers while females were identified using their brood pouches (Van Stappen, 1996b) using a hand magnifier (x 10 magnification). The total number of males and females was counted in each sample and was used to estimate percentages of either sex per treatment.

4.2.6.6. Ovoviviparous nauplii production

As soon as copulation was observed among the adult animals, appearance of nauplii was checked daily by visual inspection of samples collected from each tank using an Erlenmeyer flask. When nauplii were spotted, harvesting also commenced and was performed twice (mornings and evenings) daily. This was done manually by siphoning the entire content of each tank through a plastic container with a 500 µm mesh bottom in which adult animals were restrained allowing nauplii to pass through. The container was fitted into a detachable 125 µm mesh in which the nauplii were collected. Both the container and the detachable nets were submerged into a large plastic bowl containing water of the same culture salinity (Figure 4.4). Adults were immediately returned back into their respective culture tanks while harvested nauplii were rinsed into an Erlenmeyer flask (Figure 4.4) and allowed to stand for a few minutes for debris to settle at the bottom while nauplii swam in the water column above. The water along with the animals was then carefully passed through a separate 125 µm mesh net to concentrate the animals. Thereafter, the

daily nauplii production per tank was determined by washing the nauplii into exactly 20 mL of water and the estimated nauplii density was determined from duplicate samples of 1 mL each.

Adult animals as well as the ovoviviparously produced nauplii were also subjected to biochemical analyses to determine the composition and concentrations of amino- and fatty acids. Samples were freeze-dried before they were further analyzed (Garcia-Ortega, Verreth, Coutteau, Segner, Huisman & Sorgeloos, 1998). Procedures for the analyses were as described above.

Table 4.2: Formulae used in calculating survival and growth parameters of *Artemia* during the mass culture test

Parameter	Formulae	Description
Survival (%)	$\frac{\text{Total number of survivors}}{\text{Initial stock – total number of sampled animals}} \times 100$	
Specific growth rate (% day^{-1})	$\frac{\ln(W_t - W_0) \times 100}{T \text{ (days)}}$	ln = natural logarithm W_0 = dry weight (µg) of *Artemia* biomass at the start of experiment W_t = dry weight (µg) of *Artemia* at 9 days of culture
Feed conversion ratio (FCR)	$\frac{F}{W_t - W_0}$	F = dry weight (mg) of feed supplied during 9 days W_0 = dry weight (µg) of *Artemia* at the start of experiment W_t = dry weight (µg) of *Artemia* at 9 days of culture
Protein efficiency ratio (PER)	$\frac{W_t - W_0}{F \times P}$	F = dry weight (mg) of feed supplied to *Artemia* during 9 days of culture P = crude protein percentage in the feed W_0= dry weight (µg) of *Artemia* at the start of experiment W_t = dry weight (µg) of *Artemia* at 9 days of culture
Wet weight biomass density (gL^{-1})	$\frac{\text{Total weight – weight of empty filter}}{\text{Volume of sampled culture water}}$	

4.2.6.7. Average number of nauplii per female per day

The average number of nauplii per female per day was calculated by dividing the total number of nauplii per treatment replicate by the total number of the reproductive days. The resultant value was further divided by the average of the number of females in the replicate. The estimates for the number of females on day 21 (day of commencement of nauplii production) was also used for the calculation. Final values are presented as averages of the replicates in each treatment.

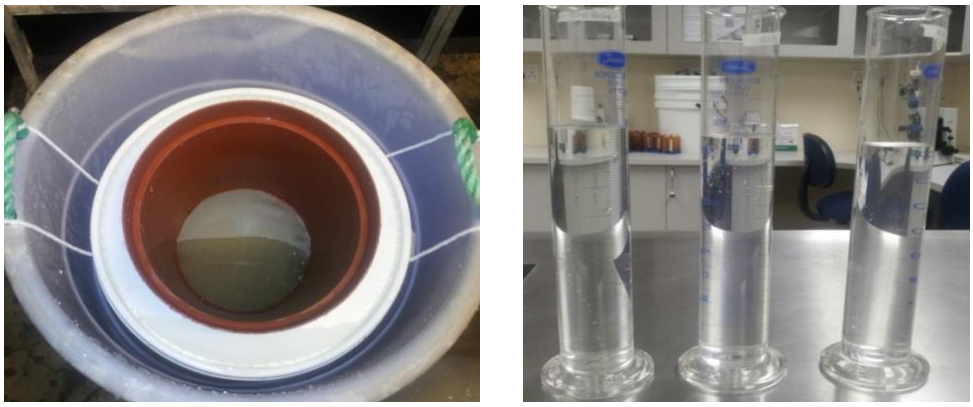

Figure 4.4: Plastic bowl consisting of detachable 500 and 125 µm mesh size containers used for harvesting adults and nauplii respectively of *Artemia* (left picture). Erlenmeyer flask used for separating nauplii from debris during harvest (right picture)

4.2.7. Statistical analysis

Data were analyzed using SAS for Windows version 9.3 and XLSTAT 2017. Assumptions of normality and homoscedasticity were tested before analyses were performed. One-way ANOVA was used to analyse the data. Analysis of Covariance (ANCOVA) was used to analyse survival and growth over time in Experiment 2. Linear regression coefficients for all treatments were calculated from the resultant parameter estimates. Where heteroscedasticity was indicated, output for Welsh's ANOVA assuming unequal variances were also obtained. Bonferroni post-hoc test was performed to identify differences between LS means. P-values of less than 0.05 were considered significant. Descriptive statistics was used to show differences in weight gain, biomass, SGR, FCR, PER, sex ratio and average number of nauplii per female measured among biomass fed stock suspensions of the different agricultural materials

4.3. Results

4.3.1. Biochemical analysis of feeds and mass-cultured *Artemia*

The proximate composition of the different feed materials (Table 4.3) showed that over 60 % of their dry matter is composed of carbohydrates except for the CP feed which had only slightly above 29 % carbohydrate, with higher levels of crude protein and lipid.

Inspite of the relatively low levels of nutrients of the different feed materials, appreciable levels of protein and their constituent amino acids, as well as fatty acids were found in the adult biomass fed with the respective feeds in experiment 2 (Tables 4.4 and 4.5, respectively). With regard to the ovoviviparous nauplii produced in experiment 2, harvested from the group fed barley pellets (BP), a similar pattern for protein and fatty acids content as seen in the parent animals was also observed in the nauplii. Nauplii harvested from the group fed oat pellets (OP) also showed similar protein

and amino acids content as their parents (fatty acids could not be analyzed). Both the adults and ovoviviparous nauplii subjected to the biochemical analyses were found to contain essential and non-essential amino acids (Table 4.4), as well as saturated, mono-unsaturated and poly-unsaturated fatty acids in their tissues (Table 4.5).

Table 4.3: Mean ± standard deviation (n=2) of proximate composition (expressed as % of dry matter) of the different experimental feed materials

Feed type	Parameters						
	Moisture (%)	Crude protein (%)	Crude lipid (%)	Carbohydrate (%)	Crude fibre (%)	Ash (%)	Gross energy ($mJ.kg^{-1}$)
*VF (Control)	10	≥30	7	-	-	-	-
CP	10.8±0.0	37.2±0.0	6.8±0.3	29.7±3.5	9.3±3.9	6.3±0.0	18.8±0.1
OP	8.9±0.1	8.4±0.1	5.0±0.2	66.2±0.5	8.8±0.5	2.8±0.0	17.4±0.0
BP	8.8±0.4	15.3±0.0	3.3±0.2	64.8±0.1	4.1±0.1	3.8±0.0	16.9±0.1
WBG	11.7±0.1	7.3±0.0	2.0±0.0	77.7±0.5	0.6±0.2	0.8±0.2	16.3±0.0
WWG	11.1±0.1	12.9±0.0	2.8±0.1	70.5±0.1	1.3±0.0	1.5±0.0	16.8±0.0

*Composition as given by supplier; - = not given. VF = Vinh Chau feed, CP = canola pellet, OP = oat pellet, BP = barley pellet, WBG = whole barley grain, WWG = whole wheat grain

Table 4.4: Amino acids composition (expressed as $\mu g g^{-1}$) in the different agricultural materials used as feed during the different experiments, in biomass fed with these different feed materials and in the ovoviviparous nauplii harvested from biomass fed with the different feed materials (n = 1)

Protein/amino acids	Treatment 1			Treatment 2			*Treatment 3		**	**
	OP	BOP	Ovoviviparous nauplii harvested from biomass fed OP	BP	BBP	Ovoviviparous nauplii harvested from biomass fed BP	WBG	BWBG	WWG	CP
Crude protein	8.34	33.34	36.13	15.28	35.25	41.31	6.82	33.72	12.01	37.22
Phenylalanine	0.28	1.26	1.33	0.43	1.45	1.64	0.45	1.21	0.41	1.11
Histidine	0.00	0.62	0.66	0.00	0.70	0.83	0.17	0.64	0.00	0.45
Isoleucine	0.26	1.26	1.41	0.47	1.41	1.59	0.25	1.32	0.39	1.35
Leucine	0.56	2.27	2.35	0.88	2.56	2.83	0.48	2.20	0.82	2.67
Lysine	0.43	2.71	2.83	0.91	2.76	3.21	0.14	2.60	0.46	3.05
Methionine	0.31	0.50	0.49	0.25	0.58	0.65	0.08	0.51	0.27	0.58
Tryptophan	-	-	-	-	-	-	-	-	-	-
Valine	0.37	1.60	1.68	0.64	1.83	2.07	0.33	1.56	0.51	1.79
Argenine	0.33	3.71	3.85	0.42	3.79	5.12	0.36	3.29	0.28	1.63
Tyrosine	0.20	1.18	1.29	0.28	1.33	1.58	0.28	1.26	0.22	0.82
Aspartic	0.66	3.34	3.64	1.39	3.73	4.43	0.30	3.50	0.62	2.75
Serine	0.33	1.72	1.80	0.50	1.87	2.16	0.30	1.67	0.52	1.50
Glycine	0.36	1.35	1.49	0.53	1.62	1.87	0.28	1.41	0.47	1.78
Glutamic acid	1.74	4.86	5.28	2.45	5.52	6.28	1.59	5.19	4.52	7.52
Threonine	0.26	1.68	1.75	0.47	1.82	2.12	0.21	1.68	0.34	1.52
Alanine	0.37	2.17	2.20	0.66	2.36	2.70	0.21	2.08	0.42	1.64
Proline	0.37	1.50	1.61	0.96	1.68	1.93	0.84	1.42	0.16	2.22
Cystine	0.00	0.23	0.25	0.00	0.27	0.28	0.03	0.27	0.00	0.21
Asparagine	-	0.02	0.08	-	0.15	0.11	-	0.09	-	-
OHproline	-	0.06	0.05	-	0.07	0.07	-	0.06	-	-
Ornithine	-	0.21	0.16	-	0.22	0.09	-	0.34	-	-
Total amino acids	6.85	32.2	34.2	11.25	35.7	41.6	6.31	32.3	11.43	32.59

OP = oat pellet, BOP = biomass fed oat pellet, BP = barley pellet, BBP = biomass fed barley pellet, WBG = whole barley grain, BWBG = biomass fed whole barley pellet, WWG = whole wheat grain, CP = canola pellet. *Ovoviviparous nauplii not analysed as nauplii production was too low; ** = Not used in mass culture test, hence no data for biomass and nauplii

Table 4.5: Fatty acids composition (expressed as % of total fatty acids) in the different agricultural materials used as feed during the different experiments, in biomass fed with these different feed materials and in ovoviviparous nauplii harvested from biomass fed with BP (n = 1)

Symbol (Name)	CP	WWG	OP	BOP	WBG	BWBG	BP	BBP	Ovoviviparous nauplii harvested from biomass fed BP
10:0	0.07	0.00	0.00	0.02	0.00	0.02	0.06	0.07	0.02
12:0 (Lauric)	0.01	0.01	0.04	0.07	0.20	0.09	0.20	0.12	0.13
13:0	0.01	0.01	0.01	0.00	0.02	0.01	0.01	0.01	0.01
14:0 (Myristic)	0.16	0.14	0.37	1.07	0.45	1.65	0.71	1.16	1.26
15:0 (Pentadecanoic)	0.14	0.11	0.02	0.24	0.15	0.42	0.32	0.52	0.53
18:0 (Stearic)	1.99	0.99	1.77	8.35	1.77	14.28	1.75	10.16	9.91
20:0 (Arachidic)	0.20	0.07	0.13	0.12	0.14	0.19	0.22	0.16	0.17
22:0 (Behenic)	0.11	0.06	0.09	0.03	0.11	0.06	0.25	0.05	0.04
24:0 (Lignoceric)	0.20	0.14	0.12	0.11	0.19	0.15	0.28	0.15	0.15
14:1	0.01	0.01	0.00	2.51	0.01	4.14	0.06	2.30	2.76
16:1 (Palmitoleic)	0.71	0.13	0.32	3.87	0.15	8.51	0.93	6.76	7.20
17:1 (Heptadecanoic)	0.11	0.06	0.01	0.22	0.00	0.32	0.19	0.56	0.60
18:1 ω 9c (Oleic)	52.43	15.84	43.13	40.87	13.64	17.05	26.64	25.95	24.91
20:1 (Eicosanoic)	0.42	0.72	0.76	0.45	0.70	0.28	0.87	0.37	0.36
24:1 (Nervonic)	0.25	0.24	0.06	0.18	0.25	0.27	0.27	0.25	0.27
18:2 ω 6c (Linoleic)	33.76	74.91	50.33	35.99	74.61	37.81	52.11	34.94	37.41
18:3 ω 6 (λ - linolenic)	0.00	0.00	0.00	0.56	0.00	1.56	0.00	0.88	0.62
18:3 ω 3 (α – linolenic)	9.08	5.53	2.57	1.36	7.08	2.68	14.19	6.52	8.01
20:2 ω 6 (Eicosadienoic)	0.13	0.20	0.09	1.17	0.32	1.50	0.25	1.47	1.25
20:3 ω 6 (Eicosatrienoic)	0.00	0.00	0.00	0.28	0.00	0.25	0.00	0.35	0.24
20:3 ω 3 (Eicosatrienoic)	0.14	0.73	0.18	0.02	0.29	0.02	0.41	0.02	0.02
20:4 ω 6 (Arachidonic)	0.00	0.00	0.00	1.92	0.01	7.33	0.00	5.77	2.97
20:5 ω 3 (Eicosapentaenoic)	0.00	0.00	0.00	0.36	0.01	0.86	0.00	1.13	0.83
22:2 ω 6 (Docosadienoic)	0.10	0.10	0.03	0.11	0.08	0.16	0.17	0.15	0.16
22:6 ω 3 (Docosahexaenoic)	0.04	0.03	0.03	0.15	0.06	0.18	0.04	0.18	0.19

CP = canola pellet, WWG = whole wheat grain, OP = oat pellet, BOP = biomass fed oat pellet, WBG = whole barley grain, BWBG = biomass fed whole barley grain, BP = barley pellet, BBP = biomass fed barley pellet

4.3.2. Screening of feed materials on a laboratory-scale (Experiment 1)

Significant effect of the feed materials on the survival (F = 26.3, P = 0.0001) and length (F = 20.6; P = 0.0001) of the animals was observed at P < 0.05 level. Highest survival percentage (81.3 %) was observed in the treatment fed a stock suspension prepared from BP, followed by those fed WBG (70.7 %) and OP (61.3 %), among which no significant difference was found (Figure 4.5). The lowest survival (8.7 %) occurred in the CP treatment. It was surprising however, that equally poor survival (8.0 %) was observed in the treatment fed with the VF (control). The final length of the animals fed with BP (5.9 ± 0.3 mm) was significantly higher than all those that received other feed types. No significant difference in length was observed between the groups fed WBG and OP, as well as between OP and WWG (Table 4.6). Due to near total mortality which occurred in some replicates that received CP and VF feed, these treatments were not included in the length analysis. Based on the results of experiment 1, only BP, OP and WBG were used in Experiment 2.

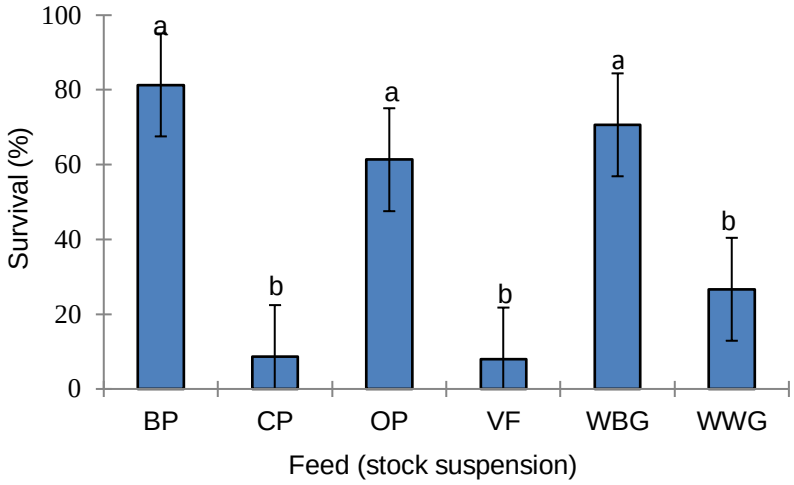

Figure 4.5: Mean survival (n=3) of Great Salt Lake *Artemia* fed 8 mL.day^{-1} of stock suspension prepared from the different agricultural materials (for abbreviations, see Table 4.4) over a nine day test in bottles (Experiment 1). Error bars corresponds with standard deviation. Different superscripts show significant difference (P<0.05) between means. BP = barley pellet, CP = canola pellet, OP =oat pellet, VF = Vinh Chau feed, WBG = whole barley grain, WWG = whole wheat grain

Table 4.6: Mean ± standard deviation values (n = 3) of final length of Great Salt Lake *Artemia* fed 8 mL.day^{-1} stock suspension prepared from the different agricultural materials (for abbreviations, see Table 4.4) over a nine day test in bottles (Experiment 1). Different superscripts show significant differences between means

	Feed types					
	BP	WBG	OP	WWG	CP	VF
Length (mm)	5.9±0.3^{a}	4.7±0.3^{b}	3.9±0.2^{bc}	3.0±0.2^{c}	nm	nm

BP = barley pellet, WBG = whole barley pellet, OP = oat pellet, WWG = whole wheat grain, CP = canola pellet, VF = Vinh Chau feed. nm = not measured due to insufficient samples

4.3.3. Mass culture of *Artemia* on a pilot-scale (Experiment 2)

4.3.3.1. Physico-chemical parameters

Frequent tank cleaning and water exchange in the mass culture system ensured that salinity and other water quality parameters such as temperature, dissolved oxygen, pH and nitrogen metabolites, were maintained within desired levels (Dhont & Lavens, 1996; Dhont & Van Stappen, 2003) (Table 4.7).

Table 4.7: Mean ± standard deviation of water quality parameters for freshly diluted culture water (n=3) and culture water during the experimental period

Description	Parameters						
	Salinity (gL^{-1})	Temperature (˚C)	pH	DO	NH_3	NO_2^-	NO_3^-
				mgL^{-1}			
Freshly diluted culture water	20.0±0.0	28.7±0.0	7.6±0.0	7.5±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Culture water (Week 1-6)	20.3±0.5	28.1±0.6	7.2±0.2	6.2±0.4	0.03±0.1	0.1±0.2	0.1±0.9

For analysis performed during the experimental period, salinity, DO, temperature and pH were checked daily (n= 42), while NH_3, NO_2^- and NO_3^- were checked weekly (n= 6). Since the system is recirculatory, all analyses were performed either directly or with samples taken from the system's reservoir depending on the parameter being analyzed

4.3.3.2. *Artemia* survival and growth

Survival among the treatments had significant difference (ANOVA; F = 313.01; P = 0.0001) during the mass culture test. Over the full test period (6 weeks), the biomass fed BP stock suspension had significantly higher survival (79.7 ± 4.6 %) than those fed with OP (69.7 ± 7.0 %) and WBG (10.0 ± 3.2 %) (Figure 4.6).

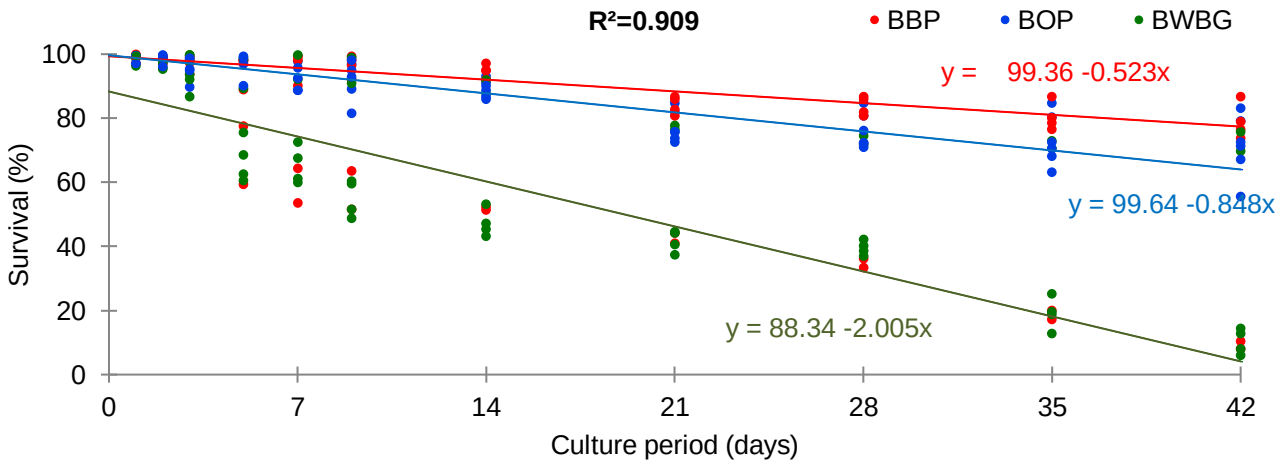

Figure 4.6: Linear regression for survival of GSL strain of *A. franciscana* fed stock suspension of the different agricultural materials during a 42 days mass culture in tanks. BBP = biomass fed barley pellet, BOP = biomass fed oat pellet, BWBG = biomass fed whole barley grain

Similarly, a significant difference (F = 13.02; P = 0.0001) was also observed in growth. Again, the biomass fed the BP stock had significantly higher growth (6.1 ± 0.8 mm final length after 9 days of culture) than those which received WBG and OP, with a length of 5.3 ± 1.1 mm and 5.1 ± 1.0 mm, respectively (Figure 4.7). Nauplii hatched from an estimated 0.25 g of cyst (estimation was done on the basis that 1 g of GSL cysts product consists of an average of 325,000 cysts (Geert Rombaut, INVE, pers. comm.) and fed with BP, OP and WBG stock suspensions were converted into an equivalent wet biomass of 5.9, 4.3 and 3.4 kg.m^{-3}, respectively, within the 9-days culture period in 0.02 m^3 of water at 28 ˚C. The growth of the BP treatment group was significantly better than either OP or WBG treatment groups which were similar. From the results of the calculated production parameters (Table 4.8), higher individual weight gain (87.5 ± 7.5 µg), feed conversion ratio (1.2 ± 0.1), protein efficiency ratio (5.7 ± 0.0) and growth rate (17.7 ± 0.4 % day^{-1}) were observed in the BP treatment. Nauplii production started on day 21 after inoculation after which daily harvest was maintained for 22 days (Figure 4.8). Within this period, an estimated (rounded to the nearest 100) total of 135,300; 118,600 and 1,700 nauplii were harvested from the biomass fed BP, OP and WBG stock, respectively. Average daily yield was approximately 6,200; 5,400 and 78, with a peak of 15,500; 10,400 and 400 nauplii, respectively. Furthermore, a higher number of nauplii per female per day were produced by the biomass fed with BP stock (51.1 ± 7.8), followed by those fed with OP (48.1 ± 6.1). The lowest value was observed in the WBG treatment (1.6 ± 0.4) where reproduction was intermittent and only lasted for a week (Table 4.8).

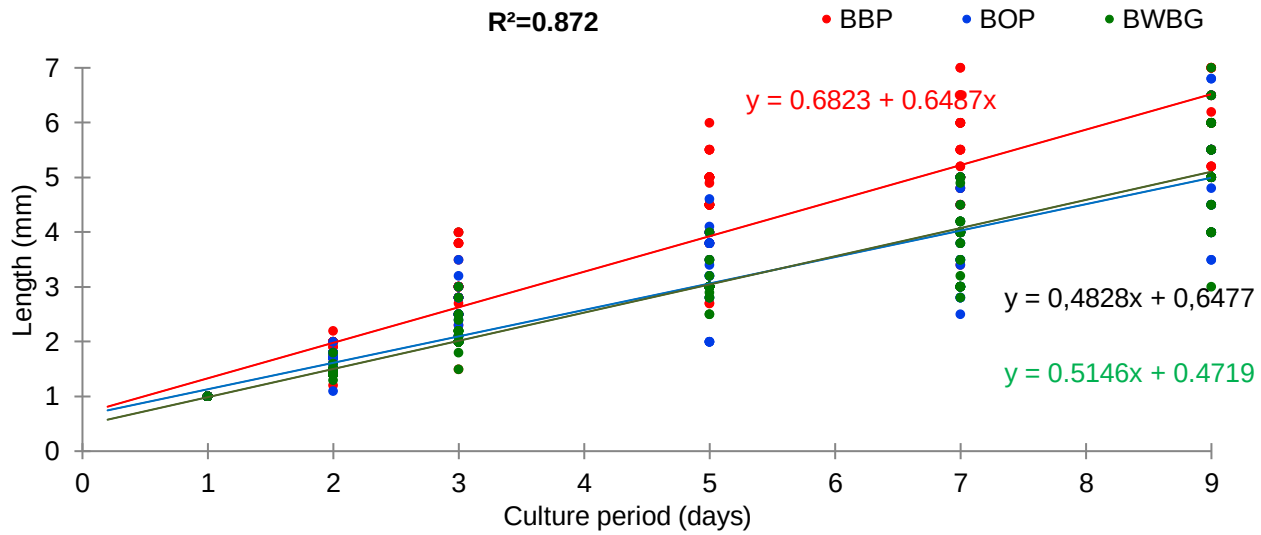

Figure 4.7: Linear regression for growth in length of GSL strain of *A. franciscana* fed stock suspension of the different agricultural materials over nine days of mass culture in tanks. BBP = biomass fed barley pellet, BOP = biomass fed oat pellet, BWBG = biomass fed whole barley grain

Table 4.8: Mean ± standard deviation (n = 2) of production parameters of GSL strain of *A. franciscana* fed a stock suspension of the different agricultural materials during the mass culture test (Experiment 2)

Feed type	Parameters							
	Weight gain (µg individual^{-1})	Biomass (g L^{-1})	SGR (% day^{-1})	FCR	PER	Sex ratio (%) M	Sex ratio (%) F	Av. number of nauplii. female^{-1}day^{-1}
BOP	75.5±7.5	15.3±1.3	16.9±0.6	2.2±0.2	5.5±0.6	39.9±1	60.1±1	48.1±6.1
BWBG	69.5±17.9	12.7±0.4	16.7±1.4	2.6±0.1	5.3±0.2	50.7±9	49.3±9	1.6±0.4
BBP	87.5±7.5	19.2±2.7	17.7±0.4	1.2±0.1	5.7±0.0	41.5±6	58.5±6	51.1±7.8

BOP = biomass fed oat pellet, BWBG = biomass fed whole barley grain, BBP = biomass fed barley pellet, SGR, specific growth rate, FCR = feed conversion ratio, PER = protein efficiency ratio, M= Males; F= Females

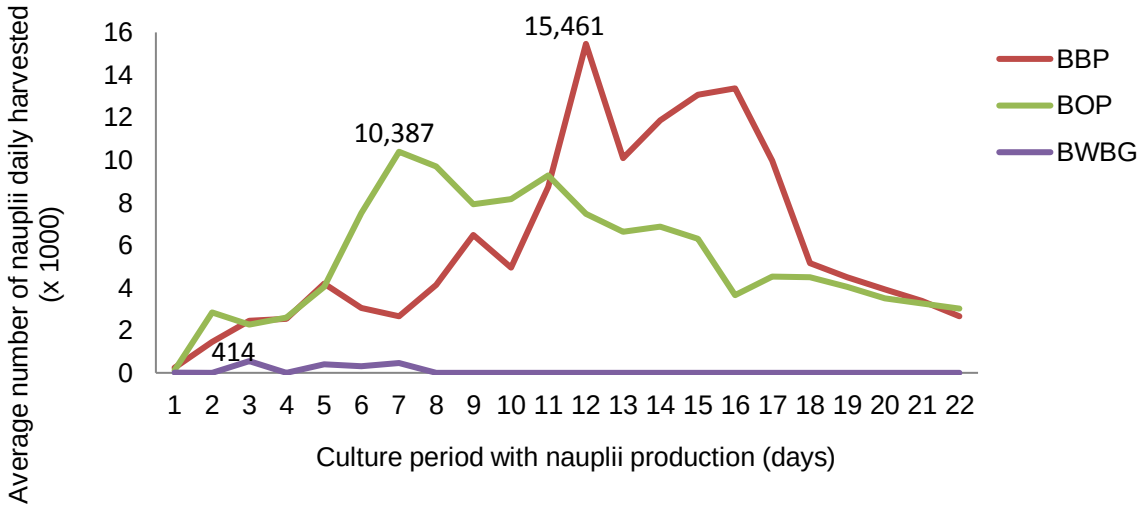

Figure 4.8: Daily nauplii production of *Artemia* biomass fed a stock suspension of different agricultural materials. BBP = biomass fed barley pellet, BOP =biomass fed oat pellet, BWBG = biomass fed whole barley grain. Values on top of peaks represent maximum daily harvest

4.4. Discussion

In nutrition of filter feeding animals, the size of the food material offered is important as it determines its ingestibility for the animal being fed (Merchie, 1996). The particle size suitable for feeding *Artemia* adults has been shown to be within the range of 2 - 50 µm (Dobbeleir *et al.*, 1980; Van Stappen, 1996b). Using the mechanical dry grinding method (Dobbeleir *et al.,* 1980) to crush the agricultural materials and manual sifting of the powder through a 50 µm mesh proved to work effectively. The fact that the guts of the animals were seen to be filled with the feed materials, coupled with the observed compact faeces within sampled culture water, indicated that the particle size met the prescribed size range for adequate intake by *Artemia*. However, the manual sifting was a slow process which was time consuming and required intensive labour. Refrigeration of the dried sifted powder ensured the use of the same batch of feed throughout the experimental period,

as it helped in maintaining quality. With regard to the quality, no specific test was conducted in order to ascertain the status of the feed materials during their storage period; however, no change was observed with respect to their texture, colour and smell. This suggests that no fungal growth occurred; hence refrigerating such feed materials could ensure their use over a considerable period of time.

Coupled with culturing the *Artemia* at low salinity, maintaining optimal ambient conditions through frequent tank cleaning, adequate water exchange and constant high oxygen levels in the system is of key importance for achieving ovoviviparity, as observed in this study and as reported by Lavens & Sorgeloos (1987). Although *Artemia* can tolerate very high levels of nitrogen metabolites (Dhont and Van Stappen, 2003), the frequent exchange of a portion of the water and the biofilter incorporated in the system ensured that these metabolites were kept at low levels which was beneficial in the overall performance of the animals.

From the outcome of the two tests conducted, it was seen that the feed materials were responsible for the differences observed in the parameters measured both for the culture in bottles and for the mass culture in the recirculation system. Generally, the BP feed consistently gave the best results among all the feeds tested. The poor results obtained with WWG during the feed screening test is consistent with the findings of Dobbeleir *et al.* (1980) who reported that wheat bran is nutritionally insufficient as mono-diet for brine shrimp culture. Given the relatively higher protein and lipid content of the control (VF) and the CP feeds, higher performance was expected; however, the contrary was observed. Although the feed materials appeared to be physically in good condition when they were used, their higher lipid content may have caused degradation as a result of oxidation. Due to delay in the commencement of the experiments, the quality of the control feed (VF) in particular (although refrigerated) may have been affected since it was supplied earlier. In a preliminary mass culture trial (not reported here), these two feed materials also appeared to affect water quality through intense foaming within a short period after start of the culture. Consequently, it was difficult to control the system as, being connected through the joint recirculation system, they also affected tanks receiving other feed types. Similar observations have been reported with soybean powder and this was attributed to the high amounts of soluble protein in the feed, which lead to high ammonia build up (Dobbeleir *et al*., 1980). Another possible reason for the poor performance, particularly with the CP feed, could be contamination with pesticides during production or storage of the product (Lavens *et al.,* 1987).

Based on the encouraging results obtained with BP, WBG and OP in the first experiment in bottles, these three feed materials were used for a more prolonged and up-scaled mass culture test, which was meant to come closer to a practical nauplii production system, such as it might be used in a hatchery. The observed values for biomass weight and length in the treatments fed the different

feeds in Experiment 2 of the present study were higher than those reported by Dobbeleir *et al.* (1980); Lavens and Sorgeloos (1987). In the report of Dobbeleir *et al*. (1980), 3.8 kg of adult biomass was produced from nauplii hatched out of 20 g cysts and a maximal length of 3.2 mm was reached in less than 2 weeks within a 2 m^3 raceway at 25 ˚C, using processed soybean powder. Similarly, they produced an average of 2 $kg.m^{-3}$ biomass within a period of 2 weeks at 28 ˚C. On the other hand, Lavens and Sorgeloos (1987) were able to convert nauplii hatched from 10 g cysts into approximately 2 kg live weight of pre-adults after 2 weeks of culture in a 1 m^3 raceway at 28 ˚C. In the present tests, nauplii hatched from 0.25 g cysts and fed with BP, OP and WBG stock suspension were converted into the equivalent of 5.9, 4.3 and 3.4 $kg.m^{-3}$ biomass, respectively, within a 9-days culture period in 0.02 m^3 of water at 28 ˚C. During the same culture period, the growth (i.e. final length) in all the groups was above 5 mm.

With regard to the individual weight gain, FCR, PER and SGR, the higher values observed with the group fed BP stock suggests that this feed was more beneficial to the animals compared to the other food types used. The survival rate (79.9 %) of BP fed animals is also comparable with 80 % reported with animals fed soybean diet (Dobbeleir *et al*., 1980). Considering the estimated average daily yield of about 6,200 nauplii from the biomass fed BP stock, and an average individual nauplius wet weight of 10.2 µg, the mass culture system has a potential of a total nauplii production of 0.38 g wet weight day^{-1} in 90 L (15 L X 6 tanks). This is far below the 30 g wet weight nauplii day^{-1} 100 L^{-1} reported by Lavens and Sorgeloos (1987). To explain the low nauplii production in the present study, the quality of the feed used will be the first to be implicated. Contrary to the mixed diet of fresh baker's yeast and an emulsified enrichment product of highly unsaturated fatty acids (HUFA) and vitamins, occasionally supplemented with a corn by-product as used by Lavens and Sorgeloos (1987), agricultural materials used as sole diets in the present study may be inadequate for achieving high fecundity. These feed materials may to a large extent affect the reproductive performance of the maternal population.

With regard to the biochemical contents, it has already been reported that brine shrimp *Artemia* are very efficient protein converters (Lavens & Sorgeloos, 1987). These authors stated that although rice bran contains only low levels of plant proteins, *Artemia* were able to biosynthesize higher levels of nutrients as 60 % of the adult dry weight was composed of animal protein rich in essential amino acids. Furthermore, when materials low in protein and lipid are used for the feeding of *Artemia*, bacteria and protozoans which develop easily in cultures can use the food as substrate to biosynthesize these essential nutrients which compensates for the deficiency in the supplied feed (Dhont & Lavens, 1996). This could be a likely scenario in this study as most of the feed types used contain a substantial (>60%) amount of carbohydrates, of which it has been shown that when added in culture tanks, it stimulates the conversion of nitrogen waste into heterotrophic bacterial mass. This in turn can be used as a nutrient source and then converted into protein-rich *Artemia*

biomass (Toi *et al*., 2013). This was illustrated in the present study by the higher levels of biochemical components found in the adults fed with the different feeds and in nauplii harvested from their biomass. The concentrations of amino acids found in *Artemia* have generally been considered not to be of a problem (Merchie, 1996). In the present study, all the essential amino acids were found to be present in the adults as well as in the ovoviviparous nauplii. However, the amounts of protein found in the adults reared with the different feed materials (OP = 33.3 %, BP = 35.3 %, WBG = 33.7 %) falls short of those observed in adult *Artemia* grown naturally (58 %) and with rice bran (60 %) (Sorgeloos *et al*., 1987). Nutritionally, the levels of protein found in the nauplii in the treatments fed OP (33.34 %) and BP (41.31 %) feed could meet favourably with the requirements for many fish species of commercial importance (Garling & Wilson, 1976; Wang, Takeuchi & Watanabe, 1985).

In practice, *Artemia* nauplii are usually required for feeding of larvae of aquatic organisms; however, most studies on nutrient requirements of fish focus on the juvenile stage (Dabrowski, 1986). This has been attributed to the ability of juvenile and adult fish to select the appropriate composition from a variety of diets in relation to their requirement for macronutrients (Sanchez-Vazquez, Yamamoto, Akiyama, Madrid and Tabata, 1999; Rubio, Sanchez and Madrid, 2003), contrary to the larvae whose capacity for such selection may not yet be developed (Hamre, Yufera, Roonestad, Boglione, Concecao & Izquierdo, 2013). Focusing on freshwater species, some estimated dietary protein requirements for maximal growth of small fish (mainly juveniles) have been put forward (NRC, 1993). Examples include the common carp *Cyprinus carpio* (31-38 %), channel catfish *Ictalurus punctatus* (32-36 %), *Tilapia zillii* (35 %) and Nile tilapia *Oreochromis niloticus* (30 %) (Ogino & Saito, 1970; Garling & Wilson, 1976; Takeuchi, Watanabe & Ogino, 1979; Mazid, Tanaka, Katayama, Rahman, Simpson & Chichester, 1979; Wang *et al.*, 1985). Although amino acids are generally not a problem in *Artemia*, sulphur amino acids such as methionine are limiting (Merchie, 1996). The pathological and growth effects caused by the deficiency in methionine in fish are critical. The amounts (expressed as percent of dry matter) for methionine found in the group fed BP (0.65) meets the requirements for channel catfish (0.6) and Nile tilapia (0.75) while the level found in OP (0.49) falls short for the same species as well as for common carp (1.2) (Wilson, Allen, Robinson & Poe, 1978; Nose, 1979; Santiago & Lovell, 1988).

Considering fatty acids, it has been highlighted that under normal circumstances, the composition in aquatic animals represents a balance between those found in the diet, those obtained from non-lipid sources through biosynthesis, and others through biosynthesis from lipid sources (De Wet, 2000). In the present study, among the fatty acids identified in the animals, considerably high amounts of oleic 18:1(ω-9), linoleic 18:2(ω-6), and linolenic18:3(ω-3) acids were particularly observed. Also, some amounts of arachidonic 20:4(ω-6), eicosapentaenoic 20:5(ω-3) and docosahexaenoic 22:6(ω-3) acids were found (although mostly in traces). Generally, similar levels of the respective fatty acids were seen in the *Artemia* adults as reported by Dobbeleir *et al.* (1980).

However, the levels found in the ovoviviparous nauplii in the present study differed from those found by these authors. They reported a lower level (5.1 %) of 18:2(ω-6), and a higher level (27.4 %) of 18:3(ω -3). These contrast with the higher (37.4 %) 18:2(ω-6), and lower (8.0 %) 18:3(ω -3) levels found in this study. It was observed that the amounts in the ovoviviparous nauplii followed the same pattern as in the parent animals. These differences may be in line with the observation that concentrations of 20:5n-3 and 22:6n-3 vary between different strains of *Artemia* as well as between batches of the same strain from the same biotope, which was traced to the biochemical content of the microalgae which the parental *Artemia* fed on (Lavens & Sorgeloos, 1991). Considering the profile of the saturated, mono-unsaturated and polyunsaturated acids, it can be seen that the concentrations found in the BP feed were higher than those found in the other feed materials tested. Although no 20:5(ω -3) and very marginal amounts of 22:6(ω-3) were found in all the feeds, the amount (14.19 %) of 18:3(ω -3) found in the BP feed was higher than that found in all the other feed materials. As highlighted earlier (in Chapter 3), the bacteria growing on the feed particles may be responsible for biosynthesizing certain nutritional compounds which may finally end up in the *Artemia* (or contribute to the nutritional profile of the *Artemia*).

The high amounts of 18:2(ω-6) and 18:3(ω-3) particularly in the ovoviviparous nauplii, suggests that the nauplii could be regarded as a good source of fatty acids especially when fed to freshwater fish species which have the ability of desaturating and further elongating these fatty acids into longer chains such as 20:5(ω -3) and 22:6(ω -3). It has been reported that in the diet, an estimated 1 % linoleic and linolenic acids, 1 % linolenic or arachidonic acids, 1-2 % linolenic acid or 0.5-0.75 % eicosapentaenoic and docosahexanoic acids and 0.5 % linoleic acid, would be required by common carp, *Tilapia zillii,* channel catfish and the Nile tilapia respectively, to yield maximal growth (Watanabe, Takeuchi & Ogino, 1975; Takeuchi & Watanabe, 1977; Kanazawa, Teshima, Sakamoto & Awal, 1980; Takeuchi, Satoh & Watanabe, 1983; Satoh, Poe & Wilson, 1989). Except for the relatively low docosapentaenoic acid value (0.19 %), all the other essential acids observed in this study are well above the levels required for the different freshwater fish species considered. Further to all the considerations and assumptions made in this study, we do not know to what extent the microflora has been biosynthesizing compounds, which finally show up in the *Artemia* and have contributed to the observations made.

4.5. Conclusion

In conclusion, it was found that not all agricultural materials which are low in nutrients can sustain the culture of *Artemia* particularly for attaining ovoviviparity. However, feed materials with nutrient levels such as the BP feed could be used to mass produce the GSL strain (*A. franciscana*) with subsequent ovoviviparous nauplii production. This feed was found to support culture with less mortality and good growth of the animals. Considering the low nauplii production level achieved, it is envisaged that when using such low-cost feed materials which are low in nutrients such as

protein and lipid, application of for example HUFA-rich enrichment products is beneficial as demonstrated in the study of Lavens and Sorgeloos (1987). These and in combination with the selected GSL strain and culture at the selected 20 gL^{-1} salinity may support increased fecundity.

Chapter 5

Ovoviviparously produced *Artemia* nauplii are a suitable live food source for the larvae of the African catfish (*Clarias gariepinus*: Burchell, 1822)

CHAPTER 5

OVOVIVIPAROUSLY PRODUCED *ARTEMIA* NAUPLII ARE A SUITABLE LIVE FOOD SOURCE FOR THE LARVAE OF THE AFRICAN CATFISH (*CLARIAS GARIEPINUS*: BURCHELL, 1822)

Redrafted after:

Richard Bwala[1, 2 & 3]; Khalid Salie[1] and Gilbert Van Stappen[2]

[1]Department of Animal Sciences, Stellenbosch University, South Africa

[2]Laboratory of Aquaculture & *Artemia* Reference Center, Ghent University, Belgium

[3]National Institute for Freshwater Fisheries Research, New Bussa, Nigeria

***Aquaculture Research,* 2018;49:3319–3328.**

Abstract

Brine shrimp *Artemia*, the most common live food organism used in larviculture, can reproduce either oviparously (production of dormant cysts) or ovoviviparously (direct production of nauplii), depending on environmental conditions. Ovoviviparous *Artemia* nauplii have seldom been considered as a source of live food in aquaculture, partly due to the convenience and the developed techniques associated with the production and use of the dormant cysts. In many countries in Africa, however, hatchery managers do not have access to a reliable supply of affordable good quality cysts. In this study, we therefore demonstrated the potential of a system designed for the continuous ovoviviparous production of nauplii at low salinity, using Great Salt Lake *Artemia franciscana* and micronized agricultural material as feed. The suitability of the produced nauplii was tested by feeding them directly to *Clarias gariepinus* larvae in comparison with oviparous nauplii and decapsulated cysts. Larvae that were fed the decapsulated cysts had a significantly higher value for final total length (18.3 ± 0.7 mm), final individual wet weight (65.2 ± 5.2 mg), final individual dry weight (17.5 ± 0.7 mg) and specific growth rate (26.4 ± 0.3 % day^{-1}) than those fed either of the live *Artemia* diets. Conversely, higher survival (100 %), better protein efficiency ratio (2.6 ± 0.1) and feed conversion ratio (1.0 ± 0.1) were observed in larvae fed with the ovoviviparous nauplii ($P<0.05$). Overall, we conclude that the ovoviviparous nauplii could serve as an alternative live food for larval fish. If optimized, the system could be validated for integration in hatcheries.

Keywords: Ovoviviparous, *Clarias gariepinus, Artemia* nauplii, live food, larvae

5.1. Introduction

Globally, *Clarias gariepinus* (Burchell, 1822), is a common aquaculture fish species belonging to the family Clariidae, genus *Clarias,* and with the widest latitudinal range (about 70 degrees latitude) (de Moor & Bruton, 1988). It is one of the most important commercial aquaculture species in Africa (Teugels, 1984; Hecht, 2013a). Currently, the total production in sub-Saharan Africa is estimated at 229,090 tonnes (FAO, 2017b). As with other commercially important fish species used in aquaculture, the success of its culture depends on a constant supply of fingerlings. This has been achieved through mass production of its larvae, which in turn relies on the good understanding of the biology and the diet requirements of the larvae. At this growth stage, the larvae are restricted to natural food, mainly zooplankton (Hecht, 2013b). Although *C. gariepinus* larvae possess a well advanced digestive system, they lack a functional stomach; consequently, the pepsin digestion provided by live food could be related to their requirement for live food (Verreth *et al*., 1993). It has been shown that live food in the form of *Artemia* nauplii, small *Daphnia, Moina* and rotifers, or an inert diet such as dry decapsulated cysts of *Artemia*, are essential for this species during the first 4 - 6 days after the start of exogenous feeding (Verreth & Van Tongeren, 1989; Awaiss & Kestemont, 1998; Enyidi *et al*., 2014). Also, it has been demonstrated that even after initial feeding with the live food, an abrupt change to feeding with formulated dry diet alone may be associated with some drawbacks such as poor growth and survival (Verreth, 1994; Chepkirui-Boit *et al.,* 2011).

Relative to other live food types, *Artemia* is the most widely used in the larviculture of many cultured fish species. This is because of the higher survival and growth commonly observed in the fed animal. Such high yields have been partly attributed to the assumption that certain proteolytic enzymes present in developing embryos and nauplii of *Artemia* aid in their digestion within the digestive tract of the predator fish (Lavens & Sorgeloos, 1996; El-Dahhar, Salama, El-Greesy & Shaheen, 2013). Additionally, the convenience with which the nauplii and decapsulated cysts of *Artemia* are used as food sources for fish and shell fish larvae has made it popular (Van Stappen, 1996a; Sorgeloos *et al.,* 2001). However, over-reliance on importation of the cysts has been identified as a bottleneck for their use in many hatcheries, especially in sub-Saharan Africa (Sorgeloos *et al.,* 2001). Furthermore, cysts of varying qualities are currently marketed, some of which have low hatchability or poor nutritional composition due to factors such as methods of harvesting, processing, storage, or type of food available to the parent populations (Hedayati & Bagheri, 2010; Vasuderan, 2012). Low availability and variable quality of commercial *Artemia* cyst products result in increased costs and unreliability of hatchery production.

As a source of live food, so far only the oviparous mode of reproduction in *Artemia* (i.e. females producing cysts when ambient conditions are stressful) has been considered as a means of supply for use in hatcheries. The controlled production of cysts has already been studied and practiced (Versichele & Sorgeloos, 1980; Lavens & Sorgeloos, 1984; Kulasekarapadian & Ravichandran,

2003; Litvinenko, Litvinenko *et al.*, 2015). Since in principle all *Artemia* strains could as well reproduce ovoviviparously (i.e. females produce live nauplii when ambient conditions are favourable) (Van Stappen, 1996b), the controlled nauplii production via this mode of reproduction has also been demonstrated for possible integration in aquaculture hatcheries (Lavens & Sorgeloos, 1987). The latter mode of reproduction was considered in the present study to serve as an alternative means for on-site production of good quality live food, when hatcheries have little access to affordable good quality commercial cysts. This also presents the possibility for continuous production and harvest of the nauplii assuming that a reproducing *Artemia* population can be maintained in a hatchery. So far, however, no study has been conducted using this *Artemia* type as live food for feeding larvae of aquaculture species. In this study, a culture system was designed for continuous production and harvest of *Artemia* nauplii from a reproducing population. The aim was to test the feasibility of direct feeding of the ovoviviparous nauplii to *C. gariepinus* larvae and the suitability of this nauplii type as live food in comparison with the conventional decapsulated cysts and oviparous nauplii.

5.2. Materials and methods

5.2.1. Protocol review, ethics clearance and experimental permits

All protocols employed in this study were ethically reviewed and approved with Protocol #: SU-ACUD15-00002 (see Chapter 4).

5.2.2. Study location, experimental design and treatments used

The study was conducted at the aquaculture facility of the Welgevallen experimental farm, Stellenbosch University, South Africa. Three *Artemia* types (all belonging to the Great Salt Lake strain of *Artemia franciscana*) including ovoviviparous nauplii (OVV), oviparous nauplii (OVP) and decapsulated cysts (DEC) were used as experimental diets and each was fed to a group of *C. gariepinus* larvae consisting of five replicates in a randomized design. Cysts of the Great Salt Lake strain of *Artemia franciscana* Kellogg 1906 (supplied by Ocean Nutrition, Belgium) were used for daily hatching of the oviparous nauplii which was done according to standard procedures described by Lavens and Sorgeloos (1996), while the decapsulated cysts (INVE, Baasrode, Belgium) were purchased from a commercial pet shop. The ovoviviparous nauplii were harvested manually twice daily from an indoor *Artemia* mass production system.

5.2.3. *Artemia* mass culture

The *Artemia* mass culture involves a production system containing a reproducing *Artemia* population cultured in the earlier described water recirculation system (WRS) unit, which was designed and monitored for the purpose of achieving nauplii production (see details in Chapter 4). The ongrowing *Artemia* population was fed with a stock suspension prepared from micronized

barley pellets. This product had shown to sustain a reproducing *Artemia* population relative to other agricultural materials used as food sources (see Chapter 4).

5.2.4. Preservation of live food

In order to maintain the quality of the respective *Artemia* live food types, they were preserved at optimal conditions until needed during each feeding period of the fish larvae. Ovoviviparous nauplii, freshly harvested from each culture tank, were always pooled before transferring into fresh 20 g L^{-1} saline water in gently aerated bottles, placed in a polystyrene box containing freshwater and stacked with ice packs to keep a low temperature around 4 $^{\circ}$C (Sorgeloos *et al*., 2001) (Figure 5.1). Although the manual method of harvesting of the ovoviviparous nauplii did not allow for strict harvest of first instar nauplii, using the cold preservation technique ensured that they were kept alive at slow metabolic rate, retarding further moulting and maximally maintaining their energy content (Sorgeloos *et al*., 2001). The same storage conditions were used for the oviparous nauplii, obtained from hatching cysts. The decapsulated cysts were kept in a refrigerator at 4 °C.

Figure 5.1: Preservation of ovoviviparous and oviparous nauplii. Harvested animals placed in a polystyrene box containing freshwater and stacked with ice packs (left picture); covered polysterene box with aeration tubes (right picture)

5.2.5. *Clarias gariepinus* larvae experiment

5.2.5.1. Culture system and water recirculation

The catfish culture experiment was set up 30 days after the start of the *Artemia* mass culture, a period which allowed nauplii production (which started at day 21) to increase and reach an appreciable daily harvest. A water recirculation system was used consisting of fifteen glass aquaria (50 cm x 9 cm x 25 cm) which served as fish rearing tanks, connected with a sump (90 cm x 32 cm x 39 cm). Each of the rearing tanks was further demarcated with 125 µm mesh screens replacing one side of the tank's wall; thus, the precise fish holding area within the tanks was 20 cm x 9 cm x

7 cm. Tap water from Stellenbosch municipal supply (kept overnight in order to reduce chlorine content that could be deleterious to the fish larvae) was used to fill the sump which was maintained at 84 L volume. The sump water was pumped continuously at a flow rate of 0.4 $Lmin^{-1}$ into the rearing tanks which were also maintained at a 5 L volume. A temperature of 28.1 ± 0.2 ˚C was maintained within the system using two 500 W submersible automatic aquarium water heaters placed into the sump pre-adjusted to the required temperature. Continuous aeration was supplied from an air compressor connected to a series of 5mm diameter rubber tubing fitted at the tip end with air stones and placed into each of the tanks.

5.2.5.2. Hatching of fish larvae, stocking, and feeding protocol

Fertilized eggs of *C. gariepinus* were sourced from a private fish farm: Aquaculture Academy, Grahamstown, South Africa. They were incubated and hatched at 28 ˚C using a 500 W submersible automatic aquarium water heater with continuous aeration supplied through an air compressor over 24 h (Potongkam & Miller, 2006). At 48 h post hatch, before yolk was completely absorbed, uniform sized larvae with an average individual initial length of 6.4 ± 0.5 mm and wet weight of 1.6 ± 0.1 mg were stocked into the glass aquaria at a density of 25 individuals per tank. The initial length and weight were determined using five replicate samples, each consisting of 25 individuals taken from the pool of excess larvae not used in the experiment. The stocked larvae were exposed to a light and dark regime of 12h:12h. Feeding commenced the next morning after stocking and it lasted for 7 days based on the protocol of Hecht (2013a). Feeding was done to satiation by offering small amounts of the respective food types during four feeding periods each day at 08:00, 11:00, 14:00 and 17:00 h (Hecht, 2013a). Satiation was determined by visually observing the larvae during feeding, and taking note of the gradual reduction in their feeding vigour until feeding ultimately stopped and they settled on the bottom of the tanks (Garcia-Ortega, Verreth, Vermis, Nelis, Sorgeloos & Verstegen, 2008). The transparency of the gastro-intestinal tract allowed visual observation of gut fullness after every feeding. The total number of the respective nauplii types fed to the fish larvae per tank at each feeding period was determined from a known volume (in mL), corresponding with a number of nauplii calculated from triplicate samples of 100 µL. As for the decapsulated cysts, weighed quantities were given and the corresponding number of cysts was calculated on the basis that 1 g of commercial GSL cyst product consisted of averagely 325,000 cysts (Geert Rombaut, INVE, pers. comm.). This was used to estimate the daily amount of nauplii or cysts eaten on average per individual larva (Table 5.1). The nauplii were rinsed with freshwater before they were fed to the fish.

Table 5.1: Mean ± standard deviation values (n = 5) of the estimated daily *Artemia* nauplii and decapsulated cysts eaten by individual *Clarias gariepinus* larvae during the 7 days feeding test

Day	Diet (*Artemia* types)		
	OVV	OVP	DEC
1	435.8±0.0	422.6±0.0	1040.0±0.0
2	616.0±0.0	580.4±0.0	1048.7±19.4
3	656.2±0.0	628.0±0.0	1586.0±35.6
4	684.4±0.0	684.4±0.0	1600.1±60.4
5	706.0±0.0	785.2±0.0	1883.3±86.7
6	854.8±0.0	836.0±0.0	2152.4±99.1
7	864.0±0.0	920.0±94.4	2841.4±235.9
Total number of nauplii and cysts	4,817.2	4,857.0	12,151.9
Dry weight of diet (mg)	10.1	12.1	37.4

OVP = oviparous nauplii, OVV = ovoviviparous nauplii, DEC = decapsulated cysts

5.2.5.3. Tank cleaning and measurement of water quality parameters

Faecal matter and other debris were removed from the fish rearing tanks every morning prior to feeding by gently siphoning culture water through 5 mm rubber tubing. Water quality parameters including temperature (˚C), dissolved oxygen (DO - mgL^{-1}) and pH were checked daily in each culture tank using an IP 67 combo multi-function waterproof meter. Ammonia (NH_3), nitrite (NO_2^-) and nitrate (NO_3^-) were measured every two days using HACH's DR/850 colorimeter following salicylate, diazotization and cadmium reduction methods, respectively. Daily, 25 % of water in the sump was exchanged with fresh municipal water which was also allowed to stand overnight to reduce chlorine content.

5.2.5.4. Fish sampling and data collection

After seven days of culture all fish larvae per tank were counted and used to determine the final total length, final wet and dry weights. Length was measured for individual larvae using a measuring board to the nearest 0.01 mm (Adewolu, Akintola & Akinwumi, 2009). Wet weight was obtained by first blotting the larvae with paper towel before they were placed in clean pre-weighed aluminium foil cups and weighed using a digital analytical balance to the nearest 0.01 mg (Verreth & Bieman, 1987). Thereafter, the wet samples were transferred into the oven at 60 ˚C for 24 h followed by an additional 4 h at 105 ˚C (Verreth & Bieman, 1987), after which they were placed in a desiccator and allowed to cool for 30 min before the moist-free samples were re-weighed following the same procedure as in wet weight determination. The averages for the wet and dry weights were calculated based on the surviving larvae per tank. The data collected were used to compute survival, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and condition factor (K) of the fish larvae in the different treatments (Table 5.2). To obtain the weight of food eaten by individual fish larvae, the total amount of nauplii eaten throughout the seven days feeding period was multiplied by the individual dry weight of the respective nauplii types (i.e. 2.5 ± 0.1 and 2.1 ± 0.1 µg for oviparous and ovoviviparous nauplii, respectively). This was calculated

from duplicate samples, each consisting of 500 nauplii which were oven dried at 60 ˚C over 24 h. As for the group fed the decapsulated cysts, the weight of cysts fed was used as such, since there was no change in weight after oven drying.

Table 5.2: Formulae used in calculating survival and growth parameters of the fish larvae after a seven day feeding period

Parameter	Formulae	Description
Survival (%)	$\frac{\text{Total number of survivors}}{\text{Initial number}} \times 100$	
Specific growth rate (% day^{-1})	$\frac{\ln(W_t - W_0) \times 100}{T \text{ (days)}}$	ln = natural logarithm W_0 = dry weight (mg) of fish larvae at the start of experiment W_t = dry weight (mg) of fish larvae at the end of experiment T = duration of experiment
Feed conversion ratio (FCR)	$\frac{F}{W_t - W_0}$	F = dry weight (mg) of feed supplied to fish larvae during experimental period W_0 = dry weight (mg) of fish larvae at the start of experiment W_t = dry weight (mg) of fish larvae at the end of experiment
Protein Efficiency Ratio (PER)	$\frac{W_t - W_0}{F \times P}$	F = dry weight (mg) of feed supplied to fish larvae during experimental period P = crude protein percentage in the feed W_0= dry weight (mg) of fish larvae at the start of experiment W_t = dry weight (mg) of fish larvae at the end of experiment
Condition factor (K)	$\frac{W}{l^3} \times 100$	W = wet weight (mg) of fish larvae l = length (mm) of fish larvae

5.2.6. Proximate and amino acid analyses

Crude protein and amino acids were analysed, respectively in triplicate and on single samples, of the three *Artemia* types at the central analytical laboratory of Stellenbosch University. The moisture content of the two nauplii types was also determined. All other values presented are given by suppliers. Crude protein was determined following the Dumas method using LECO FP 528 for the

quantitative determination of nitrogen in samples (AOAC, 2002) and calculation was performed using the conversion factor of 6.25. Moisture was determined by loss of water when drying at 100 ˚C for 24 h (AOAC, 2002). Amino acids were measured in solutions after hydrolysis of proteins using standard 6M HCl acid digestion (AOAC, 2003). This analysis involves the standard Waters method using an Ultra C_{18} 2.1 x 100mm x 1.7µm column and derivatizing agents [6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC)]. After dilution and derivatization, amino acid separation and detection was performed using a Waters Acquity Ultra Performance Liquid Chromatography (UPLC) fitted with a photodiode array (PDA) detector.

5.2.7. Statistical analysis

Data were analysed using SAS for Windows version 9.3. The assumptions of normality and homoscedasticity were tested before analyses were performed. A one-way ANOVA was used to compare the effect of the three different *Artemia* types on the survival, final length and weight, specific growth rate, food conversion ratio, protein efficiency ratio and condition factor of *C. gariepinus* larvae. Least square means pairwise comparisons were performed using Bonferroni post-hoc tests for all the analyses. P-values of less than 0.05 were considered significant.

5.3. Results

5.3.1. Water quality parameters

Using the described *Artemia* mass production system, daily harvest of the nauplii was achieved. Salinity in the *Artemia* nauplii production system was maintained at the desired level. Other water quality parameters such as temperature, dissolved oxygen, pH and nitrogen metabolites in the *Artemia* mass culture system were maintained within favourable ranges (Dhont & Lavens, 1996; Dhont & Van Stappen, 2003) (see Chapter 4). For the *C. gariepinus* larvae culture, the ranges of the respective parameters favourable for the species were also maintained (Potongkam & Miller, 2006) (Table 5.3). No significant difference was observed between the mean values for temperature, dissolved oxygen (DO) and pH between the treatments over the entire culture period (P>0.05).

Table 5.3: Mean ± standard deviation values of water quality parameters analyzed during the culture of *Clarias gariepinus* larvae

Temperature (˚C)	pH	DO (mgL^{-1})	NH_3 (mgL^{-1})	NO_2^- (mgL^{-1})	NO_3^- (mgL^{-1})
28.1±0.2	7.1±0.2	7.0±0.2	0.04±0.04	0.01±0.01	0.04±0.03

5.3.2. Proximate and amino acid analyses

The protein concentration in the ovoviviparous nauplii (41.3 %) was found to be lower than in the other *Artemia* types with oviparous nauplii having the highest value (55.9 %) (Table 5.4). The

concentrations of the essential amino acids were generally lower in the ovoviviparous nauplii than in the decapsulated cysts and highest in the oviparous nauplii (Table 5.5). The moisture content of the ovoviviparous and oviparous nauplii was similar and was well above the amount in the decapsulated cysts.

Table 5.4: Proximate composition for the three *Artemia* types used as feed for the larvae of *Claria gariepinus*

Artemia type	Parameters				
	Protein (%)	Lipid (%)	Ash (%)	Fibre (%)	Moisture (%)
OVV	41.3	-	-	-	80.0
OVP	55.9	11.0	5.0	-	80.2
DEC	54.0	9.0	4.0	6.0	5.0

- signifies either not analysed or not given by suppliers. Protein composition (dry weight basis) for the three *Artemia* types was analyzed in the present study. Moisture content (wet weight basis) for ovoviviparous and oviparous nauplii was also analyzed. All other values are given by suppliers. OVP = oviparous nauplii, OVV = ovoviviparous nauplii, DEC = decapsulated cysts

Table 5.5: Composition and concentrations (% in dry sample) of amino acids in the three different *Artemia* types used as feed for larvae of *Clarias gariepinus*

Amino acids composition	Diet (*Artemia* types)		
	OVV	OVP	DEC
Phenylalanine	1.64	2.14	2.00
Histidine	0.83	1.15	0.96
Isoleucine	1.59	2.37	2.15
Leucine	2.83	3.73	3.73
Lysine	3.21	4.70	4.40
Methionine	0.65	1.04	1.18
Tryptophane	-	-	-
Valine	2.07	2.61	2.59
Arginine	5.12	6.50	3.91
Threonine	2.12	2.52	2.51
Aspartic acid	4.43	4.85	4.64
Serine	2.16	2.88	3.66
Glycine	1.87	2.35	2.26
Glutamic acid	6.28	6.96	7.56
Tyrosine	1.58	2.01	1.55
Alanine	2.70	3.22	2.47
Proline	1.93	2.50	2.00
Cystine	0.28	0.27	0.19
Asparagine	0.11	0.26	-
OH proline	0.07	0.02	-
Ornithine	0.09	0.03	0.01

- = Not detected; OVP = oviparous nauplii, OVV = ovoviviparous nauplii, DEC = decapsulated cysts

5.3.3. Survival and growth parameters

All the catfish larvae were observed to feed with high vigour even when fed the ovoviviparous nauplii, which had a pale appearance compared with the other *Artemia* types which were orange in colour. Cannibalism and malformations were not observed among the fish larvae throughout the experimental period. Survival of the animals, though generally high (> 90%), was significantly higher ($P<0.05$) when feeding either type of nauplii than when feeding decapsulated cysts (Table 5.6). The larvae in the group fed ovoviviparous nauplii grew to a final average individual length of 16.2 ± 0.1 mm which was not significantly different from those fed the oviparous nauplii ($P>0.05$). But the highest length was observed in the group fed cysts (18.3 ± 0.7 mm) and this value was significantly higher ($P<0.05$) than when feeding either type of nauplii. A similar trend was observed for the wet weight values of all the three groups (Table 5.6). Individuals in the group fed ovoviviparous nauplii showed a significantly lower dry weight (11.0 ± 0.5 mg) compared to the group fed oviparous nauplii (12.3 ± 0.5 mg). The feed conversion ratio for the group fed ovoviviparous nauplii (1.0 ± 0.1) did not differ significantly ($P>0.05$) from the oviparous group, but was better than when feeding decapsulated cysts (2.2 ± 0.1). Protein utilization observed in the ovoviviparous group (2.6 ± 0.1) was significantly higher than in the other treatments ($P<0.05$). The condition factor showed an intermediate value when feeding ovoviviparous nauplii, which was not significantly different from either of the other food types (Table 5.6).

Table 5.6: Mean ± standard deviation values (n = 5) for survival and growth parameters of *Clarias gariepinus* larvae fed three different *Artemia* types for seven days. Different superscripts show significant differences between means in a row

Parameters	Diet (*Artemia* types)			ANOVA
	OVP	OVV	DEC	P-value
Survival (%)	96.8 ± 3.4^{a}	100.0 ± 0.0^{a}	90.4 ± 4.6^{b}	0.002
Final total length (mm)	16.9 ± 0.3^{b}	16.2 ± 0.1^{b}	18.3 ± 0.7^{a}	0.0001
Final individual wet weight (mg)	44.7 ± 1.8^{b}	42.0 ± 1.7^{b}	65.2 ± 5.2^{a}	0.0001
Final individual dry weight (mg)	12.3 ± 0.5^{b}	11.0 ± 0.5^{c}	17.5 ± 0.7^{a}	0.0001
Specific growth rate (% day^{-1})	24.2 ± 0.2^{b}	23.5 ± 0.3^{c}	26.4 ± 0.3^{a}	0.0001
Food conversion ratio	1.0 ± 0.0^{b}	1.0 ± 0.1^{b}	2.2 ± 0.1^{a}	0.0001
Protein efficiency ratio	1.8 ± 0.0^{b}	2.6 ± 0.1^{a}	0.9 ± 0.0^{c}	0.0001
Condition factor (K)	0.9 ± 0.1^{b}	1.0 ± 0.0^{ab}	1.1 ± 0.1^{a}	0.004

Specific growth rate, food conversion ratio and protein efficiency ratio were calculated on dry weight basis while K-factor was calculated on wet weight basis; OVP = oviparous nauplii, OVV = ovoviviparous nauplii, DEC = decapsulated cysts

5.4. Discussion

Clarias gariepinus is recognized as one of the major fish species cultured in the sub-Saharan region of Africa. Live food such as *Artemia* is important for rearing of the larvae from the start of exogenous feeding (Verreth, 1994; Enyidi *et al*., 2014). Globally, many regions including sub-

Saharan Africa depend heavily on importation of *Artemia* cysts for larviculture. Production efficiency has thus been adversely affected through associated high costs and reduced profit margins, thereby reducing the competitiveness of aquaculture in these regions. Despite this, there is potential for the expansion of aquaculture, which underscores the need for a localized means of supplying *Artemia*. In this test, nauplii production was achieved using an *Artemia* mass culture system, operated on a cheap feed, for a sustained period of daily harvest stretching up to 22 days. Coupled with culturing the *Artemia* at low salinity, optimal ambient conditions such as maintaining constant high oxygen levels and frequent culture water exchange in the system are of key importance for achieving ovoviviparity, as observed in this study and as reported by Lavens & Sorgeloos (1987). Although *Artemia* can tolerate very high levels of nitrogen metabolites (Dhont and Van Stappen, 2003), the frequent exchange of a portion of the water and the biofilter incorporated in the system ensured that these metabolites were kept at low levels which could also be beneficial in the overall performance of the animals.

According to Merchie (1996), the effectiveness of a food organism is determined firstly by its ingestibility. The observed vigour with which the fish larvae fed on the ovoviviparous nauplii during feeding periods indicated that this nauplii type was also well accepted like the other two types of *Artemia*. Linked with the carnivorous feeding habit of *C. gariepinus*, the absence of cannibalism in all the treatments including the ovoviviparous group was a further indication of acceptance of this nauplii type by the larvae. Although the nutritional requirements of the African catfish larvae are not fully known, Hecht (2013a) highlighted that the species has a propensity toward a relatively high protein requirement. A crude protein level of 55 % and 2.5 % methionine has been recommended to promote optimal growth and body condition (Uys & Hecht, 1985). In the present study, the level of crude protein found in ovoviviparous nauplii (41.3 %) was substantially lower, whereas those of oviparous nauplii (55.9 %) and the decapsulated cysts (54 %) were approximately the recommended level. However, the ovoviviparous nauplii which had a lower protein level of 41.3 % resulted in a significantly higher protein efficiency ratio than the other two food types, suggesting that the protein in ovoviviparous nauplii may be of higher quality which led to better utilization by the fish larvae. Considering the amino acids composition, both the essential and non-essential acids found in the ovoviviparous nauplii appear to be similar to those found in oviparous instar I nauplii reported in the work of Garcia-Ortega *et al.* (1998). The absence of tryptophane in all the *Artemia* types was not surprising as this essential amino acid is usually not detected in such an analysis due to degradation by acid hydrolysis (Aragao, Conceicao, Dinis & Fyhn, 2004). But tryptophane has been reported to be related to skeletal malformation (scoliosis) in fish larvae (Cahu, Infante & Takuechi, 2003). Non-occurrence of malformation of the larvae in any of the treatments may indicate that all the *Artemia* types including the referenced ovoviviparous nauplii contained some amount of tryptophane sufficient to maintain good body condition at least for the 7-days culture period. The methionine levels in the three *Artemia* types used fall below the

recommended level of 2.5 % for catfish larvae (Uys & Hecht, 1985), and it was particularly lower in the ovoviviparous nauplii (0.65 %). These observed low levels conform to the assertion by Lavens and Sorgeloos (1991), that methionine is usually limiting in *Artemia*. Conversely, De Wet (2011) highlighted that limiting essential amino acids such as methionine and phenylalanine can be replaced up to 60 % and 50 % by non-essential amino acids such as cystine and tyrosine, respectively, hence reducing their requirement in fish larvae. Furthermore, non-essential amino acids play a significant role in saving energy that could have been used for the synthesis of essential amino acids (De Wet, 2011). Non-essential amino acids are well known to be present in decapsulated cysts and oviparous nauplii (Lavens & Sorgeloos, 1996). Their presence in ovoviviparous nauplii as observed in this study may suggest the similarity of this *Artemia* type with the oviparous type in terms of their qualitative nutritional composition. In this regard, it is notable that traces of asparagine and OH proline found in the ovoviviparous and oviparous nauplii were completely absent in the decapsulated cysts.

Focusing on the performance of the fed animals, the generally high survival which was observed in the present study supports earlier studies that reported high survival of larvae fed with either decapsulated cysts, oviparous nauplii or their combination (Verreth *et al.,* 1987; Bardocz, Kovacs, Radics & Sander, 1999; Chepkiruit-Boit *et al*., 2011). The very high survival in the group fed with the ovoviviparous nauplii suggests that the diet could serve as a good source of live food for the first feeding catfish larvae. In terms of growth, results from earlier reports indicate that fish larvae may grow more with decapsulated cysts than with the oviparous nauplii of *Artemia* (Lim, Cho, Dhert, Wong, Nelis & Sorgeloos, 2002; Adewolu *et al.,* 2009; Abubakar, Ipinjolu, Hassan & Alegbeleye, 2013). The present study also conform to this as most of the growth parameters measured were better with the decapsulated cysts treatment. However, this phenomenon may not necessarily be related only to the nutritional status of the different *Artemia* types but could also be linked to other factors such as the behaviour of the food types within the culture tanks. *Artemia* are physiologically unable to survive in freshwater for more than about an hour (Merchie, 1996). Feeding high numbers to fish larvae may not necessarily mean that all the nauplii have been eaten. From experience in this study, the fish larvae would take up the nauplii quickly while still within the water column and they refrain from eating once they are full. Non-consumed nauplii would thus die and sink to the bottom of the tank, where they quickly decompose. Conversely, a significant portion of non-consumed decapsulated cysts would sink to the bottom of the tank within few minutes after feeding. However, the cysts appear to stay intact for a longer period, allowing the larvae to feed on them later. Therefore, feeding high numbers of nauplii at once to fish larvae may thus lead to partial starvation of the larvae if feeding is not done frequently. This could result in less growth and wastage of the food. On the other hand, the larvae fed with the cysts may have had availability of food within the tank for a longer period which consequently may lead to more growth. The quality of the nauplii as food is also determined by its developmental stage at the moment of

supplying it to the fish larvae (Merchie, 1996). Freshly hatched or spawned instar I nauplii would lose their nutritional quality as they metamorphose into the second instar stage, affecting their dietary value for fish larvae (Merchie, 1996).

Comparing the present results with other studies where *C. gariepinus* larvae were fed with oviparous nauplii and/or decapsulated cysts, it was found that irrespective of the *Artemia* type used in the present study, the final total length (Table 6) of the fish larvae measured at seven days was more than the highest values of 13.8 and 9.0 mm recorded after six and 20 days of culture of Bardocz, Kovacs, Radics & Sandor (1999) and Mukai and Seng Lim (2011), respectively. The wet and dry weight values were also higher than 26.1 and 4.3 mg, respectively, obtained by Bardocz *et al.* (1999) after a six day feeding experiment with vitamin C enriched, dried and freshly decapsulated cysts as well as hatched nauplii. But the wet weight value was similar to those observed by Garcia-Ortega, Verreth, Van Hoornyck & Segner (2000) who reported values of 50.4 and 47.8 mg for larvae fed oviparous nauplii and decapsulated cysts, respectively, after the same culture period of seven days. However, the dry weight values at day seven were similar to those recorded at day nine (17.8 and 12.5 mg for larvae fed oviparous nauplii and decapsulated cysts, respectively) by Garcia-Ortega *et al.* (2000).

Regarding food utilization, it appears that the ovoviviparous nauplii were used by the larvae of *C. gariepinus* as efficiently as the nauplii obtained from cyst hatching, and better than the decapsulated cysts, as shown by the values for food conversion and protein conversion ratio (Table 6). The lower protein and amino acid levels as well as the lower dry weight found in the ovoviviparous nauplii could be reflective of their reduced nutritional quality resulting from the harvest methodology adopted in the experiment. Since moulting from the instar I phase to instar II of the nauplii occurs within 8 h (Van Stappen, 1996b) with a consequent reduction in nutritional value (Merchie, 1996), harvesting the *Artemia* tanks twice daily may not have allowed the collection of only the freshly produced instar I nauplii. The period between the daily harvests could have been sufficient for a substantial fraction of the produced nauplii to moult into the instar II stage before they were used as food. This may have affected their success as food relative to the other food types used.

5.5. Conclusion

Summarizing, the results obtained with the ovoviviparous nauplii do not compare favourably to the other *Artemia* types used in this and other similar studies. However, the ovoviviparous nauplii can serve as a valuable alternative starter diet for the larvae of *C. gariepinus*. Considering the difficulties in having easy and reliable access to affordable cysts in Africa, improvement and optimization of the *Artemia* production system may lead to its validation as an alternative to imported decapsulated or non-decapsulated cysts.

Chapter 6

Comparative economic analysis of ovoviviparously produced *Artemia* nauplii versus imported decapsulated cysts: experience of catfish hatchery operators in Kainji Lake Basin, Nigeria

CHAPTER 6

COMPARATIVE ECONOMIC ANALYSIS OF OVOVIVIPAROUSLY PRODUCED *ARTEMIA* NAUPLII VERSUS IMPORTED DECAPSULATED CYSTS: EXPERIENCE OF CATFISH HATCHERY OPERATORS IN KAINJI LAKE BASIN, NIGERIA

Abstract

Live fish food is regarded as essential particularly for the first feeding larvae of species such as Clariids. Previously, nearly all hatcheries in Africa depended on imported *Artemia* cysts. However, the soaring foreign exchange rate has grossly affected the importation, consequently resulting in the low availability and increased price of this product. Within the scope of the present study, a system was developed for the purpose of ovoviviparous naupliiproduction as an alternative to the use of nauplii hatched from purchased *Artemia* cysts. This production system uses low salinity as culture medium in order to induce *Artemia* biomass into the ovoviviparous reproductive mode. The economic viability of using the ovoviviparously produced nauplii at the current production capacity of the developed system versus the use of the imported *Artemia* cysts was analyzed. Cost analysis of producing an individual *Clarias gariepinus* larva fed with the ovoviviparously produced *Artemia* nauplii was found to be 2.15 USD while the cost involved in producing a fish larva fed with locally bought imported decapsulated *Artemia* cysts was approximately 0.002 USD. The result of the analysis shows that at the current capacity of the developed nauplii production system, the cost of feeding an individual *C. gariepinus* larva is far higher than when the imported decapsulated cysts are utilized. However, considering the high fecundity of *Artemia* with a capacity of reproducing up to 300 offspring every 4 days in optimal conditions, it is possible to achieve a higher production of the nauplii if the potential of the system is fully exploited. An estimated cost of approximately 0.00053 USD will then be used for feeding a larva of *C. gariepinus* at the same cost of producing the nauplii. By ratio, only 0.27:1 (nauplii/cysts) will therefore be expended in feeding a single larva. It was also argued that the overall benefits of using the ovoviviparous nauplii should not only be anchored on monetary terms but also on the associated benefits such as high survival rate, feed conversion ratio, protein efficiency ratio as observed in the present study. Other potential benefits also include post-larval quality of the fish, which still needs investigation, and more controllable, guaranteed and known quality of the live food product at a relatively fixed price.

6.1. Introduction

Within the sub-Saharan region of Africa, Nigeria is located in the Western part with a high human population currently estimated to reach about 180 million people (FAO, 2017b). Presently, Nigeria is among the leading fish producing countries in Africa with a production of 1,027,058 tonnes as at 2015 (National Bureau of Statistics, 2017). The fisheries sector (including aquaculture) in Nigeria contributes 3 – 4 % to the country's Gross Domestic Product (GDP) and 50 % of the population's animal protein intake (FAO, 2017b). Moreover, it is also generating employment and income to a significant number of the population (FAO, 2017b). Despite this, a production deficit of 1,444,752 tonnes has been estimated for 2015 (FDF, 2007 cited by Emmanuel, Chinenye, Oluwatobi & Peter, 2014). Recent evidence affirms this prognosis as this deficit has increased to 2.2 million tonnes (FDF, 2017), which underscores the need for increase in production. Presently, the Clariids, particularly *Clarias gariepinus* are the major and most commonly cultured fish species in the country. The production level of *C. gariepinus* reached 253,898 tonnes by 2012 (AU-IBAR, 2013). This could be attributed to the appreciable level of success achieved in the supply of catfish seeds from hatcheries which are mostly operating at small or medium scale. However, this success is experiencing a setback due to the shortage or complete absence in the supply of starter food such as live nauplii of *Artemia* or inert decapsulated cysts, which are regarded as essential particularly for the first feeding larvae of the clariid species.

Previously, nearly all hatcheries depended on imported *Artemia* cysts. However, the soaring foreign exchange rate has grossly affected the importation, consequently resulting in the low availability and increased price of this product (Maldonado-Montiel *et al.*, 2003). This has forced many of the hatcheries to resort to other, cheaper and more available, alternatives such as the formulated microdiets (Hecht, 2013a). But these alternative diets have generally been considered as less effective (in terms of performance) in the hatchery rearing of larvae of African catfish (Adewolu *et al.,* 2009). In order to ensure good quality supply of the fish larvae, there is a need to intensify the search for local means for live food production. Within the scope of this project, the continuous production of *Artemia* nauplii was considered as a potential means for the supply of live food for direct feeding to the fish larvae. This has led to the development of a nauplii production system which uses low salinity as culture medium for the purpose of inducing *Artemia* biomass into the ovoviviparous reproductive mode. In addition to the biological and zootechnical aspects of this production system, addressed in the previous chapters of this thesis, it was also considered imperative to conduct an analysis on the economic viability of using the ovoviviparously produced nauplii at the current production capacity of the developed system. Therefore, the unit cost of producing a *C. gariepinus* fish larva in Nigeria, fed with locally produced ovoviviparous *Artemia* nauplii, was compared to the cost involved when imported commercial decapsulated *Artemia* cysts are used.

6.2. Materials and methods

6.2.1. Cost analysis of fish larvae fed with ovoviviparously produced *Artemia* nauplii

An estimate was computed for the amount of nauplii produced, based on the current technical and biological knowledge of the developed nauplii production system and the quantity of the nauplii eaten by an individual *C. gariepinus* larva (Table 6.1). Nauplii yield was calculated as the average value of the daily harvest during the entire nauplii production period (see Chapter 4). Four days was used in the analysis as the standard period in which *C. gariepinus* larvae are exclusively fed on live food. Thus, the quantity of ovoviviparous nauplii eaten by an individual fish was calculated as the sum of the nauplii eaten during the first four days of the feeding experiment with *C. gariepinus* larvae (see protocol in Chapter 5). Yield of the ovoviviparous nauplii was also expressed in wet weight for the purpose of comparison with the decapsulated cysts (providing equal wet weight as live food: either as nauplii or as decapsulated cysts). The total cost involved in the production of the nauplii was calculated as the sum of all relevant expenditure, both on investments (fixed cost) and recurrent costs (variable cost). Since the information for the comparison with the use of the decapsulated cysts was collected from a field survey conducted in Nigeria, the estimates for the fixed and recurrent costs for the nauplii production system were also based on the prevailing rates (prices) in Nigeria. For sake of convenience, the costs are presented in United States Dollar (USD) with the exchange rate at the time of this report being 360 Nigerian Naira (NGN) to a dollar. A straight line depreciation method was used to compute the annual depreciation of assets on the fixed items, which means that the total cost of an asset was evenly distributed throughout its expected lifespan in order to obtain the annual cost.

Table 6.1: Production estimate of ovoviviparous nauplii using the developed production system, the quantity of nauplii eaten by an individual *Clarias gariepinus* larva and the number of fish larvae that could be fed during the exogenous feeding period (production data obtained in Chapters 4 and 5)

Size of nauplii production tanks	Number of tanks	Total volume used (L)	Average nauplii yield (harvested ind.day^{-1})	Wet weight of ind. nauplius (µg)	Total wet weight of harvested nauplii day^{-1} (g)	Number of ovoviviparous nauplii eaten by an ind. fish larva in 4 days	No. of fish larvae that could be fed (on the basis of total wet weight nauplii day^{-1}) in 4 days
(37 cm x 26 cm x 25 cm)	6	90	36,898	10.2	0.38	2,392	15.6

6.2.2. Cost analysis of fish larvae fed with imported decapsulated *Artemia* cysts

As for the fish larvae fed with the decapsulated cysts, data were obtained from hatchery operators in Kainji Lake Basin, Nigeria. Kainji Lake Basin is the first man-made Lake in Nigeria which is located on the Niger River, Niger state in western Nigeria (Figure 6.1), and it supplies a local fishing industry. It has a surface area of 1270 km^2, length of 134 km and a maximum width of about 24 km (Bankole, Yem & Olowosegun, 2011). It lies between 9^o 50’ and 1^o 55’N latitude and

between 4º 23' and 4º 45'E longitude (Olokor, 2011). A semi-structured questionnaire was used which employed a multistage sampling technique. The first stage was the delineation of Borgu emirate within the Kainji Lake Basin. The second stage was the selection of four districts in the emirate (which include Bussa, Kigera, Sabuke and Koro) that have a number of hatchery operations. Ten respondents were randomly picked from the list of 35 hatchery operators which formed the sample size for the study at the third stage (Table 6.2).

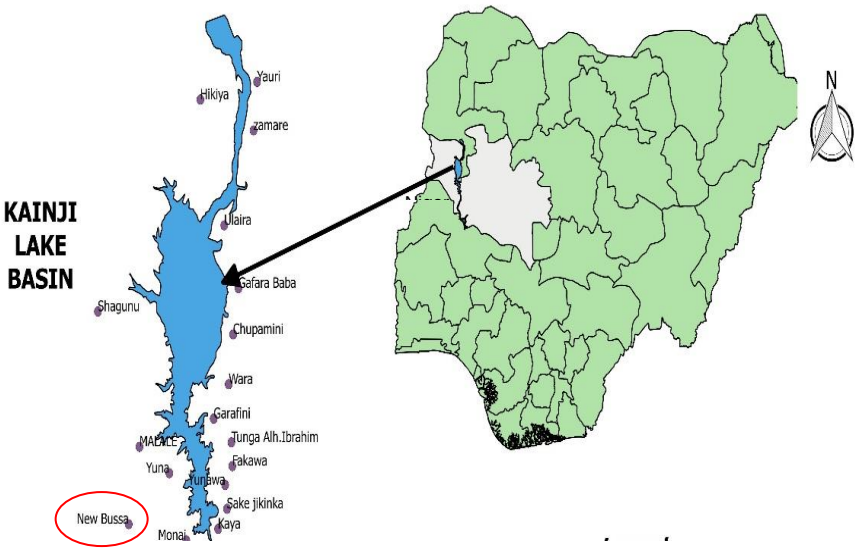

Figure 6.1: Map of Nigeria showing the study area (Kainji Lake Basin). Source: Sanni (2018)

Table 6.2: Sample of hatchery operators by district in the study area

Study area	Sampled Emirate	Selected districts	No of hatchery operators by district	No of respondents sampled
Kainji Lake Basin	Borgu	Bussa	19	5
		Kigera	4	1
		Sabuke	9	3
		Koro	3	1

6.3. Results

6.3.1. Cost analysis of fish larvae fed with ovoviviparously produced *Artemia* nauplii

6.3.1.1. Estimated fixed cost for production of the ovoviviparous nauplii

The estimated total fixed cost (TFC) of relevant inputs that was incurred in the production of the ovoviviparous *Artemia* nauplii was 79.31 USD (Table 6.3). Among the relevant inputs, the construction of the recirculation system (25.37 USD), purchase of heater (21.69 USD) and air compressor (14.46 USD) constituted the bulk of the fixed cost items representing 61.52 %. Items which had low cost include harvesting bowls (0.30 USD), feed mixer (1.51 USD) and accessories for aeration which include air stone, control valves and hose (2.17 USD).

Table 6.3: Estimated fixed costs of relevant inputs used for ovoviviparous nauplii production. Costs are based on purchase prices in Nigeria, and on production data obtained in Chapter 4.

Fixed inputs	Life expectancy (years)	Unit/Initial Cost (USD)	Annual depreciation Cost^{-1} (USD)	Weekly cost during nauplii production (USD)	*Cost during 3 weeks period for nauplii production (USD)	% of TFC
Construction of recirculation system	10	194.44	19.44	8.46	25.37	31.99
Mesh nets	5	69.44	6.94	3.01	9.04	11.40
Biomass rearing tanks	5	16.67	1.67	1.45	4.34	5.50
Water storage tank	10	83.33	8.33	0.14	0.42	0.53
Harvest bowls	10	13.89	1.39	0.10	0.30	0.38
Heaters	5	83.33	8.33	7.23	21.69	27.35
Air compressors	5	55.56	5.56	4.82	14.46	18.27
**Oxygen fittings	5	8.33	0.33	0.72	2.17	2.74
Feed mixer	1	27.78	2.78	0.50	1.51	1.90
Total		552.77	54.77	26.45	79.31	100.00

*3 weeks was the experimental period during which daily nauplii production was observed
**Aeration fittings include air stones, control valves, air tubes. TFC = Total fixed cost

6.3.1.2. Estimated variable cost for production of the ovoviviparous nauplii

The estimated total variable cost (TVC) of relevant inputs that was incurred in the production of the ovoviviparous nauplii was 58.19 USD (Table 6.4). The cost of electricity (16.30 USD), labour (hired/family) (20.83 USD), filtered sea water (10.19 USD) and municipal water (10.19 USD) represented 98.83 % of the total variable cost.

Table 6.4: Estimated variable costs of relevant inputs used for ovoviviparous nauplii production. Costs are based on purchase prices in Nigeria, and on production data obtained in Chapter 4

Variable inputs	Unit/initial cost (USD)	Weekly cost during nauplii production (USD)	*Cost during 3 weeks period of nauplii production (USD)	% of TVC
Filtered sea water (month^{-1})	13.89	3.40	10.19	17.51
Feed material (agricultural by-product)	4.17	0.17	0.51	0.88
Processing of feed material, packaging and storage	5.56	0.06	0.17	0.29
[1]Electricity costs (month^{-1})	22.22	5.43	16.30	28.01
[1]Municipal water costs (month^{-1})	13.89	3.40	10.19	17.51
[2]Labour (month^{-1})	20.83	6.94	20.83	35.80
Total	80.56	19.40	58.19	100.00

*3 weeks was the experimental period during which daily nauplii production was observed. [1]Monthly average rate charged according to units/volume of electricity/water consumed. [2]Hired and family labour whereby the two are considered to be the same and based on average number of person-hours day^{-1}

6.3.1.3. Total cost for production of the ovoviviparous nauplii

The total economic cost of producing 1.52 g (0.38 g x 4 days) of ovoviviparous nauplii which can feed 64 fish (approximately 16 fish larvae x 4 days) larvae was 137.5 USD.This was based on the feeding protocol used for feeding fish larvae with ovoviviparous nauplii: see Chapter 5; four days is the period in which live food is essential for the feeding of *Clarias gariepinus* larvae (Table 6.5).

Table 6.5: Total cost for production of the ovoviviparous nauplii

Cost items	Value (USD)	% of TC
TFC	79.31	57.68
TVC	58.19	42.32
TC	137.5	100.00

TC= Total cost, TFC= Total fixed cost, TVC= Total variable cost

6.3.1.4. Unit cost of fish larva fed with ovoviviparously produced *Artemia* nauplii

In order to determine the cost of production per larva of *C. gariepinus* using ovoviviparous nauplii as live food, the total cost of inputs (fixed cost and variable cost) used in the production of the nauplii was divided by the total quantity of fish larvae which can be fed with the quantity of the nauplii produced, thus:

$$\sum = X_1 + X_2 / Q \qquad (1)$$

where:

$\sum$ = Summation of costs used in production of ovoviviparous nauplii

X_1 = Total fixed cost (TFC)

X_2 = Total variable cost (TVC)

Q = Total quantity of fish larvae which can be fed with the ovoviviparous nauplii at the present production level of the system.

Since the estimate of the total cost of producing a number of nauplii sufficient to feed 64 *C. gariepinus* larvae was 137.5 USD, the cost of producing an individual larva is 2.15 USD.

6.3.2. Cost analysis of fish larvae fed with imported decapsulated *Artemia* cysts

6.3.2.1. Total cost involved in using imported decapsulated *Artemia* cysts

The fish larvae were fed for an average of 16 days with 3.0 kg of *Artemia* cysts which cost 36.05 USD kg^{-1}. On the average, labour (183.70 USD) and purchase of *Artemia* (108.15 USD) as variables constituted the dominant cost items, consuming 51.37 % and 30.24 % respectively of the total variable cost for the fish larvae producers (Table 6.6). The study further revealed that 30 % of the hatchery operators in the area procure their decapsulated *Artemia* cysts from places outside of their hatchery locations covering a distance ranging from 500 – 1100 km.

Table 6.6: Cost analysis for feeding 205,000 fish larvae for 16 days with imported decapsulated *Artemia* cysts. n = 10

Items of cost	Mean cost (USD)	% of TC
Purchase of *Artemia*	108.15	30.24
Transportation	33.92	9.48
Storage	14.81	4.14
Labour (family/hired)	183.70	51.37
Electricity	11.85	3.32
Fuelling	5.19	1.45
Total	357.62	100.0

TC = Total cost

6.3.2.2. Unit cost of fish larva fed with imported decapsulated *Artemia* cysts

In order to determine the cost of production per fish larvae using decapsulated *Artemia* cysts, the total cost of all inputs used in the course of production was divided by the total quantity of fish larvae produced, thus:

$$\sum_{n=1}^{n} = X_1+X_2\ldots\ldots X_n/Q \qquad (2)$$

where:

$\sum_{n=1}^{n}$ = summation of all inputs used in weaning fish larvae

X_1 = Decapsulated *Artemia* cysts used within the period

X_2 = Transportation

X_3 = Storage cost

X_4 = Labour (family / hired)

X_5 = Electricity

X_6= Fuelling

Q = Total quantity of fish larvae produced and fed with *Artemia*

From Equation 2, the total of all operating costs i.e. total variable cost (TVC) used in the course of producing a batch of 205,000 fish larvae was 357.62 USD. Therefore, on the average, it costs approximately 0.002 USD to produce a fish larva fed with locally shopped imported decapsulated *Artemia* cysts.

6.4. Discussion

With the global increase in human population, coupled with more demand for animal protein and a high deficit in fish production, many countries especially in the sub-Saharan region need to increase fish production in order to maintain the current supply and to meet projected requirements. However, this is hampered by the low supply or unavailability of fish seed (Megahed & Aly, 2009). Within the sub-Saharan region, Nigeria is one of the major fish producing countries that is faced with this challenge (Enyidi *et al.*, 2014). The fishery sector in Nigeria is characterized majorly with the production of clariid species, particularly *C. gariepinus*. For the purpose of obtaining good quality larvae of this species, live food is an essential diet during the start of their exogenous feeding (Verreth, 1994). Currently, there is dependency on imported decapsulated *Artemia* cysts, which are fed as such, without hatching, but the soaring foreign exchange rate has consequently resulted in the low availability and increased price of this product. In the present thesis, the need to find local ways for the production and supply of live food led to the development of an *Artemia* nauplii production system which uses low salinity as culture medium for the inducement of the ovoviviparous reproductive mode in *Artemia* biomass. This section presents an economic appreciation of using the ovoviviparously produced nauplii at the current production capacity of the developed system.

The result of the analysis shows that at the current capacity of the developed nauplii production system, the cost of feeding a single *C. gariepinus* larva was far higher when the produced nauplii were used compared with the use of imported decapsulated cysts. Even at the depreciated values for the various inputs used in the production of the nauplii, an estimated 2.15 USD is required to feed a single *C. gariepinus* larva compared to only 0.002 USD when the imported decapsulated cysts are used. This also means that the expenditure involved in the two forms of feeding is in the ratio of 1,075:1. Furthermore, the 16 days feeding with decapsulated cysts as practiced by the hatchery operators is much too long. If the fish larvae were fed for just four days with cysts

(analogous with feeding with nauplii, and much more in line with what the fish really need) the figure would be even more to the advantage of using decapsulated cysts, rather than nauplii.

The present results showed that the nauplii production system performed below expectation. Considering the high fecundity of *Artemia* with a capacity of reproducing up to 300 offspring every four days in optimal conditions (Van Stappen, 1996), it is possible to achieve a higher production of the nauplii if the potential of the system is fully exploited. In order to have an idea of a more optimal nauplii production and the corresponding cost of producing a single larva using these nauplii, the following assumptions were made. At a survival rate of 79.7 % (i.e. 59,775 individuals) recorded by the end of the reproductive period observed, and with female proportion of the biomass reaching 58.5 % (i.e. 34,968 individuals), and assuming that 50 % (17,484) of the females produce up to 100 nauplii per day per female, an estimated total of 629,424,000 nauplii (6,420.1 g) could be harvested within four days. Attaching a 1 % loss from harvest, a net quantity of 623,129,760 (6,355.9 g) nauplii will be obtained. This should be sufficient for feeding about 260,461 larvae. Substituting the number of the larvae into equation 1, approximately 0.00053 USD will then be the estimated cost for feeding a single larva of *C. gariepinus* at the same cost of producing the nauplii. By ratio, only 0.27:1 (nauplii/cysts) will therefore be expended in feeding a single larva.

It is important to note that the cost of production will change depending on the scale of operation. The variable costs which could change include inputs such as labour and consumables used such as feed, filtered sea water and municipal water. This means that if the system is operated at a larger scale, the cost of production will equally increase. However, increased output in terms of nauplii harvest can also be expected. Moreover, operating the system over a longer duration as a source of nauplii to feed successive batches of first-feeding catfish larvae, will also result in lowering production costs.

6.5. Conclusion

When comparing the cost of using ovoviviparous nauplii to that of decapsulated cysts for feeding of fish larvae, it is also important to note that estimating the benefits that would accrue from using ovoviviparous nauplii as live food should involve factors that enhance many important production parameters such as larval growth, survival rate and post-larval quality. Accordingly, the overall benefits of its usage should not only be anchored on monetary terms but also on these associated benefits. This means that farmers who are willing to adopt the use of the ovoviviparous nauplii would be willing to incur the initial investment cost for establishing the production facility. In other words, the attributes that inherently define the benefits of using ovoviviparous nauplii, compared to other live foods such as the decapsulated cysts of the brine shrimp *Artemia*, would entice the prospective clients. Thus, it is assumed that a market value of the ovoviviparous nauplii is a reflection of its superior characteristics such as the post-larval quality. Furthermore, and as

potential asset of the nauplii production system, it is likely to have a more controllable, guaranteed and known product at a relatively fixed price, whereas the quality and price of the imported decapsulated cysts may vary as a function of external factors that are out of the control of the fish farmer.

Chapter 7

General discussion and conclusions

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

7.1. Introduction

Aquaculture in Africa has developed considerably. This has been attributed to the emergence and intensification of small and medium scale enterprises (NEPAD, 2013). Among the areas being given attention to boost the production is the supply of sufficient and quality fish seed. The development and success in this activity is in turn dependent on the supply of quality larval food.

At present, the larviculture industry in Africa is nearly completely dependent on imported *Artemia* cysts for either hatching into nauplii for feeding as live food or direct feeding as decapsulated cysts to fish larvae. The high foreign exchange viz-a-viz increases in the cost of operation when using the imported *Artemia* cysts has led many hatcheries in this region to resort to artificially formulated feed, which has been regarded as a less efficient larval food. Conversely, the use of other live food types such as freshwater zooplankton is not popular among hatchery operators in Africa. This is partly due to their low reproductive capacity and the techniques involved in the production of suitable food (algae) required for their culture, which makes the production procedures difficult. Thus, their production and use are seldom considered in hatcheries within this region. Since live food is considered the best for larval production of most cultured species, the search for alternative ways of their supply to augment food sources for hatchery use in this region is therefore imperative. Contrary to freshwater zooplankton, *Artemia* females have been reported to have high fecundity reaching up to 300 cyst or nauplii every four days in optimal culture conditions. It is also known that in principle, all *Artemia* species possess two reproductive modes, switching between oviparity (cyst production) and ovoviviparity (live birth of nauplii) depending on ambient conditions. This offers the opportunity for controlled production of either the cysts or nauplii depending on the purpose for production. The former mode of reproduction has so far been intensely studied and *Artemia* cysts are presently produced at commercial scale and sold as off-the-shelf product in many parts of the world. On the contrary, knowledge on nauplii production through the alternative ovoviviparous mode is still limited. In the present research, the ultimate goal is the development of an on-site semi-continuous nauplii production system as an alternative live food source. So far, the technique for achieving ovoviviparity in *Artemia* has only been demonstrated through the maintenance of high oxygen level, adequate culture water exchange and diet manipulation. While incorporating some of these culture conditions, and in order to further explore into more techniques for the production, this thesis considered other aspects such as the choice of the appropriate *Artemia* strain, the use of low salinity as a culture medium and the use of low cost agricultural materials as sole diets (Chapters 3 and 4). The applicability of the ovoviviparously produced nauplii for direct feeding of one of the important cultured species (i.e. *Clarias gariepinus*) in the sub-

Saharan region was also tested (Chapter 5). The use of low salinity as a culture medium was inspired based on the hypothesis that lower salinity could limit osmoregulatory stress compared to higher salinity medium which is known to cause stress in cultured species (Wedemeyer, 1996), whereby in *Artemia,* it induces cyst production. Low cost agricultural materials were considered in order to demonstrate if locally sourced food materials could be used for the ovoviviparously reproductive population bearing in mind the fact that the production costs of the system need to be low, and that *Artemia* are known to be particulate, non-selective filter feeders and have been shown to feed on a wide range of food sources (Provasoli & Shiraishi, 1959; Barker-Jorgensen, 1966; Dobbeleir *et al.,* 1980; Dhont & Lavens, 1996). In order to determine the effect of low salinity on the survival, reproductive and life traits of *Artemia,* as well as the effect of different low cost agricultural materials as sole diets on the survival, growth and the performance of the ovoviviparously reproducing population, this study posed the following questions:

1. Can low salinity culture medium be used to achieve survival and ovoviviparity in *Artemia,* and for which strain(s)? (Chapter 3).
2. Can agricultural materials be used as sole diets for the rearing of the ovoviviparously reproductive population of *Artemia*? (Chapter 4).

As for the applicability of the produced nauplii, the questions posed were:

3. Are ovoviviparously produced *Artemia* nauplii suitable as live food for *C. gariepinus* larvae? (Chapter 5).
4. What is the production cost of these nauplii, in comparison with the more conventional type of *Artemia* used to feed catfish larvae? What is the cost implication of rearing an individual *C. gariepinus* larva using either of the live food types? (Chapter 6).

7.2. Survival, reproductive and life traits responses of four *Artemia* strains in low salinity

Being a euryhaline species, *Artemia* has high tolerance for salt stress reaching up to 300 g L^{-1} (De Vos, Van Stappen, Sorgeloos, Vuylsteke, Rombauts & Bossier, 2019). However, high survival reaching up to 90 % is often recorded at relatively lower salinities ranging from 15 g L^{-1} to 120 g L^{-1} (Sung, Pineda, MacRae, Sorgeloos & Bossier, 2008). Furthermore, *Artemia* is known to be a hypo-osmoregulator (Bradley, 2009) maintaining its body fluid at 15 g L^{-1}. Hypo-osmoregulation involves keeping body fluids hypo-osmotical to the saline environment by means of active ion transport (Bradley, 2009). Considering the need for *Artemia* to maintain its body fluids at about 15 g L^{-1}, a high salinity environment may require a substantial amount of energy to achieve this important physiological activity (Wedemeyer, 1996). Hence, culturing at low salinity would be more beneficial.

The hypothesis that culturing of *Artemia* at low salinity would generally result in good survival and ovoviviparity was tested, and the outcome showed high survival in most of the salinities and higher ovoviviparity in at least one of the media used. The differences in the observations made between

the different low salinities, however, revealed that even within the range tested, there were still influences of salinity on particularly the reproductive performance of the cultured *Artemia*. This suggests that not all low salinities are suitable for use as culture medium for achieving ovoviviparity as hypothesized. Salinities lower than 20 g L^{-1} could sustain a high survival rate of the tested strains over an appreciable period but it appears that at 5 g L^{-1}, the medium was not suitable for the purpose of reproduction. The reproduction at 10 g L^{-1} was considerable, and the performance at this salinity and those higher conforms to the field observation of Agh *et al.* (2007) whereby a salinity of 10 g L^{-1} supported the maturity of a parthenogenetic *Artemia* population with reproduction starting when salinity reached 15-20 g L^{-1}. With respect to the strain factor, it was observed that different strains of *Artemia* respond differently to the effect of low salinity, which is consistent with the observation of Vanhaecke *et al.* (1984). In their study, significant differences in the response of 13 strains to lower salinities (5 - 15 g L^{-1}) revealed that some strains such as the Buenos Aires (Argentina) *Artemia persimilis* had higher survival than others.

Several adaptive repertoires such as the water replacement hypothesis (WHR), vitrification (Crowe, Hoekstra & Crowe, 1992; Crowe, Reid & Crowe, 1996; Crowe, Carpenter & Crowe, 1998; Crowe, Clegg & Crowe, 1998), artemin protein (De Graaf, Amons & Moller, 1990) , intra-cellular pH switch (ΔpH_i) (Busa & Crowe, 1983; Crowe, Crowe, Drinkwater & Busa, 1987), stress protein (e.g. p26, Hsp70 and 90 families) (Clegg, Willsie & Jackson, 1999; Liang & Macrae, 1999), accumulation of trehalose, glycerol and guanosine (5') tetraphospho (5') guanosine (Gp_4G) (Warner, 1992), have been shown to play significant roles in the maintenance of the integrity of the diapause-destined embryo of *Artemia.* Traceable levels of the activities of similar repertoires have also been reported in the early (instar I) larval stage, which may support its survival in the face of sudden exposure to varying environmental conditions when hatching from cysts or being released as free swimming nauplii by a female *Artemia* (Clegg & Conte, 1980; Watts, Yeh & Henry, 1996; Chavez, *et al*., 1999). On the other hand, many of these adaptive repertoires are not traced at other growth stages of *Artemia*. This implies that these latter growth stages in *Artemia* could be influenced to a higher extent by external environmental conditions. Salinity influence on the osmoregulatory mechanism of the adult stage confirms this (Holliday, Roye & Roer, 1990). Since the culture medium in the present study is saline, the physiological process of primary importance is the osmoregulation. In the larvae of *Artemia*, the organ responsible for osmoregulation is mainly the salt gland. The development and possibly the degeneration of this gland have been linked with the product of a homeobox gene, APH-1; of which its expression is also restricted to the gland (Chavez, Landry, Loret, Muller, Figueroa, Peers, Rentier-Delrue, Krauskopf & Martial, 1999). The degeneration of the salt gland in young immature stages gives rise to the osmoregulatory system in adults involving chiefly the metepipodites. The enzyme responsible in osmoregulation is the Na^+/K^+ -ATPase. Increases (independent of environmental salinity) in the level of this enzyme have also been shown in the emerged embryo up to the instar I developmental stage (Lee & Watts,

1994). To our knowledge, the occurrence of this enzyme in growth stages between the instar I and the adult stages have not been reported. The occurrence of this enzyme was not measured in our study, but the results showed high nauplii survival in the tests using instar I and II in the axenic test, as well as in the larval and pre-adult stages in the xenic test. The influence of ambient salinity on the adult *Artemia* as reported by Holliday, Roye & Roer (1990), was also observed in our study; however, this was only at the lowest salinity (5 g L^{-1}) tested. Survival at higher salinities tested did not show any significant difference between the larval stage and eight day old pre-adults. Since our tests were conducted in a low salinity medium, the enzyme ornithine decarboxylase (ODC) may have played a significant role in regulation of polyamine synthesis (Watts *et al*., 1996). Polyamines have been implicated in various basic cellular processes including responses to altered cellular osmolality (Watts *et al*., 1996).

7.3. Performance of Great Salt Lake strain *Artemia franciscana* fed with low cost agricultural materials as sole diets

Results obtained during the feed screening in glass bottles and the observations made during preliminary mass culture in tanks indicated that feed materials with high lipid content could yield poor result when used in tanks. Although the chemical analysis of the commercial Vinh Chau feed and the micronized canola pellet showed that their nutrient concentrations were relatively higher, yet, significantly lower survival was obtained than for other feeds which were used in the feed screening test. Also, during the preliminary mass culture trial, it was further observed that these two feed types excessively produced foams, hence skimming off a significant amount of the stocked nauplii, especially in the first few days of the culture. Additionally, the oily status of the feed affected the culture water by covering the entire surface with a thin film of oil, with consequent quick deterioration of the culture water quality. As all tanks were connected through a joint recirculation system, this also affected other tanks receiving other feed types. Poor growth and sudden mortality was prominent in tanks fed with the Vinh Chau feed and the canola pellet feed. From these observations, it appeared that using an oily feed source to culture *Artemia* in tanks may not be suitable.

The concentration of nutrients in the adult biomass fed the different materials and in the ovoviviparously produced nauplii was well above the levels found in the feed materials used. This could be explained through interaction between microorganisms such as bacteria and carbohydrate based substrates as seen in high amount in the feed materials used. *Artemia* has already been shown to be efficient nutrient converters even when fed with low nutrient feed materials (Sorgeloos *et al*., 1980). Importantly, bacteria have been shown to contribute to *Artemia* nutrition (Toi *et al*., 2013). Bacteria and other microorganisms use carbohydrates (e.g. sugars, starch and cellulose) as food, to generate energy and grow, i.e., to produce proteins and new cells (Avnimelech, 1999). In a study with low algal (nutrient source) feeding regime, bacterial growth

was stimulated using organic carbon supplied as sucrose or soluble potato starch and ^{15}N labelled inorganic nitrogen supplied as $NaNO_3$ (Toi *et al.*, 2013). These authors reported that carbohydrate addition in culture tanks may be used to stimulate the conversion of nitrogen waste into heterotrophic bacterial mass. This in turn can be used as a nutrient source and then converted into protein-rich *Artemia* biomass. During their 15-day study, significantly improved biomass production was obtained in treatments with low algae and supplemented by carbohydrate addition. Additionally, ^{15}N accumulation and fatty acids analysis in *Artemia* indicated that *Artemia* utilized more bacteria in algal limited conditions (Toi *et al*., 2013). In an earlier study (Intriago and Jones, 1993), it was reported that *Artemia* grown to the pre-adult stage and fed with a *Flexibacter* strain of bacteria in combination with algae resulted in the best growth and biomass. The study suggested that the bacteria did not only act as food, but also assisted in the digestion of the algae which yielded high concentration of PUFAs per dry weight of *Artemia.*

In order to enhance ovoviviparity, diet manipulation has been suggested (Lavens & Sorgeloos, 1987). Although in the study of Versichele and Sorgeloos (1980), food source was not demonstrated to influence the mode of reproduction in *Artemia* among the factors examined (i.e. cyclic low oxygen tension, addition of ferric-EDTA, salinity and food source), the low harvest of nauplii in the present study may be indicating that when agricultural materials are used as sole diets for reproducing *Artemia* biomass, they may not be efficient enough to support high fecundity. In the study of Versichele and Sorgeloos (1980), no difference was observed between different algal sources and micronized rice bran when exposed to non-cyst inducing culture conditions. However, the exposure of the animals to cyst inducing conditions (i.e. low oxygen tension and addition of Fe-EDTA) resulted in a similar shift in the reproductive mode with the two feed sources. The differences observed among the different food sources in the present study may thus be as a result of the differences in chemical composition of the food sources and their interactions with the microbial community that may be present within the culture media and the food sources.

Pigment deficiency in *Artemia* feed appears to support ovoviviparity (Lavens & Sorgeloos, 1987). These authors reported that the absence of pigment in the feed may be restraining the *Artemia* from producing the haem-pigment which is an essential component in the oviparous reproduction mode. In this regard, the whitish coloration of the micronized feed (particularly the barley and the oat pellet) may have influenced the inducement of ovoviviparity observed in the present study. Additionally, it is likely that the non-pigmentation in the micronized feed could also be the reason for the observed pale pigmentation of the adult biomass and consequently in the produced nauplii.

Although low salinity supported ovoviviparity as seen in the present study, microorganisms such as some species of *Vibrio* are pathogenic and could easily be established. From our experience, it becomes practically impossible to establish a culture once the system is infected. However, it was

also found that cultures flourish easily when good hygiene is maintained. In order to minimise the occurrence of infection in such biomass culture, it is important that the system should be set up separately from other hatchery facilities e.g. fish culture units. Additionally, the frequent cleaning of tanks during the culture period is important for minimizing bacterial infection. More importantly, the entire system must be disinfected after every production cycle. The production cycle is herein referred to as a period from the start of culture to the phase when nauplii production drops significantly. Flushing the system over 24 h with a high dose of hypochlorite mixed in water with subsequent 48 h rinsing/neutralizing the effect of the hypochlorite using aerated freshwater appears to work well for suppressing pathogenic microbes in the system. Disinfection, although beneficial, may not prevent a re-colonization of the system within a short period (Qi, 2016); therefore, this author recommended that disinfection of aquaculture systems is done in combination with probiotics to ensure that beneficial rather than potentially pathogenic bacteria dominate the bacterial community (Qi, 2016). This is linked to the K-selection strategy which refers to an approach for the management of the microbial community that allows steering of host–microbiota interactions toward mutualism (Vadstein, Attramadal, Bakke, & Olsen, 2018). This results in different water microbiota with less opportunists, whereby the animal cultivated in water inhabited by K-selected microbiota perform better (Vadstein *et al*., 2018). With adequate microbial management such as in the biofloc technology, other internal compounds (apart from their nutritional value) such as the short chain fatty acids could serve as bio-control agents against pathogenic diseases (De Schryver, Crab, Defoirdt, Boon & Verstraete, 2008). In this regard, it was reported that the application of 20 mM of butyric acid (as was the case with formic, acetic, propionic or valeric acid) to the culture water of *Artemia franciscana* resulted in the protection of these organisms against pathogenic *Vibrio campbellii* (Defoirdt, Halet, Sorgeloos, Bossier & Verstraete, 2006).

During production or storage of farm products, pesticides and other chemicals possibly used may contaminate the materials rendering them a less ideal source of feed for *Artemia* production (Sorgeloos *et al*., 1980). Antinutritional factors are other possible sources of contamination in such feed materials. It is not known to what extent possible contamination with these compounds, if any, could have contributed to the low fecundity in the reproducing population of *Artemia* in the present study. In order to sort this out, it is imperative to conduct a full-fledged chemical analysis so as to determine the occurrence of toxins and/or pesticides in materials before use as feed for production of *Artemia*.

On the practical side, although the method used to obtain the micronized powder of the tested agricultural materials ensured that the particle size of a substantial portion was within the required range for easy uptake by *Artemia,* the procedure for manual sifting through the 50 µm mesh net

was quite challenging. This process may not be readily adapted in practice as it was tedious and time consuming, so it should be modified for future upscaling and routine application.

7.4. Ovoviviparously produced *Artemia* nauplii are a suitable live food source for the larvae of the African catfish (*Clarias gariepinus*)

The suitability of ovoviviparously produced *Artemia* nauplii as live food is also an important aspect relevant for the potential application of the production system. The results obtained from the feeding test with the ovoviviparous nauplii showed for the first time that they could serve as alternative to the oviparous nauplii and decapsulated cysts used in the present study and in other studies. The pale coloration of the ovoviviparously produced nauplii did not negatively affect their uptake by the fish larvae. Also, despite the lower protein content of the ovoviviparously produced nauplii, better protein efficiency and food conversion ratio was found in the fish larvae fed the nauplii. This suggested that the ovoviviparous nauplii can serve as a valuable starter diet for the larvae of *C. gariepinus*. Considering the difficulties in having easy and reliable access to affordable cysts in Africa, improvement and optimization of the *Artemia* production system may lead to its validation as an alternative to imported decapsulated or non-decapsulated cysts. For starting cultures of a reproducing population with subsequent continued harvest of the ovoviviparous nauplii, only a limited quantity of cysts will be needed compared to the daily and sometimes variable hatching of cysts associated with the oviparous nauplii. Consequently, this will reduce the high quantity of cysts that may be required for hatching into nauplii as live food or for direct feeding in the form of decapsulated cysts to fish larvae. With the current effect of climate change on natural biodiversity whereby the sustainable supply of *Artemia* cysts cannot be guaranteed (Lavens and Sorgeloos, 2000), these findings could be of interest to stakeholders in the larviculture industry especially hatchery operators. The study on controlled ovoviviparous nauplii production could lead to an alternative means of supplying live fish food in order to meet or supplement local hatchery demands.

Maintenance of the ovoviviparous nauplii production system requires that it is operated for a while to get the *Artemia* to sexual maturity (e.g. two to three weeks). Once the *Artemia* start reproducing, the system is expected to operate for a further two to three weeks before water quality problems possibly set in, the population degenerates and could eventually crash, after which one has to start anew. By implication, it therefore means that the system probably will not be much longer productive than three weeks in total, within a total lifetime of the animals of four to six weeks. Practical organisation also depends on how the hatchery operates: how frequently (e.g. per year) do they have fish larvae that need *Artemia* for four days (period during which live food is essential for the fish larvae). If the hatchery would have semi-continuously batches of freshly hatched fish larvae throughout the year or at multiple occasions, it is more efficient to try to operate an *Artemia* production system nearly continuously and to maintain the reproducing *Artemia* population as long

as possible, even over successive *Artemia*generations, because it is a lot of work to start with the culture and to get the *Artemia* to sexual maturity. If the hatchery has fish larvae batches to be fed just once or twice per year, it would start with the setup of the *Artemia* production system e.g. two weeks ahead of fish eggs hatching, in order to ensure availability of nauplii at the time that the fish larvae start feeding. In this case there will be no need to continue with the *Artemia* production system after the fish larvae have been switched to formulated diet.

This pilot study shows that while it is technically and biologically possible to produce ovoviviparous nauplii which could be used as substitute for the imported decapsulated *Artemia* cyst, the production system still requires optimization before it can reach its full potential and possibly be considered for acceptance and use at a larger scale. This was evident from the very high cost of producing fish larvae using the ovoviviparous nauplii, compared to the lower price at which the hatchery operators currently work in producing larvae using imported *Artemia* cysts. As the focus of the study was to experimentally test the feasibility for the production of the *Artemia* nauplii and subsequently undergo up-scaling, it is envisaged that when the system is eventually used at its full potential, it could serve as a viable way of supplying *Artemia* nauplii as live food to local hatchery operators in the sub-Saharan region of Africa. Due to the high cost on importation which is associated to tax rates in this region of Africa, fish feed prices are high, causing difficult access to live food, such as the *Artemia* decapsulated cysts, for larval production. For example, in Kenya, the cost of raising one catfish larva using decapsulated cysts ranges between 3 to 5 Kenyan shilling (KES), which is equivalent to an average of 0.04 USD (Abdoul, 2015), at an exchange rate of 100.95 KES to a USD. This is higher compared to the estimated 0.002 USD that the hatchery operators in Nigeria expend (Chapter 6). Despite the high cost of using the imported *Artemia* cysts, it is however evident that hatchery owners are aware of the importance of using the live food for feeding their fish larvae. The experience of some of the hatchery operators in Nigeria travelling long distances to procure their cysts confirms this. This may also suggest that factors such as variability in cyst quality have an influence on their decision for the choice of where to purchase the cysts, irrespective of the costs. In Kenya and Uganda, there are hatcheries where people buy non-decapsulated cysts and hatch them. Needless to say, when the costs needed to buy (often very expensive) non-decapsulated cysts, hatching tanks and other hatching equipment, and the procedures for the hatching itself are all put into perspective, coupled with the fact that there is often a lot of ignorance on how to do the optimal hatching, this alternative of obtaining live food is economically less attractive, and thus the nauplii production system compares more favourably with it.

When up-scaling of the nauplii production system is to be considered, the question which is likely to be posed is: will there be a benefit in economy of scale, i.e. do procedures get cheaper if the system is scaled up? This most likely should be the case for both fixed and variable costs.

Assuming a nauplii production system having basins 10 times bigger (150 L) than the tanks of 15 L each used in the present study, nauplii yield will not automatically be 10 times higher, even at the same *Artemia* density as in the small system; a bigger system may be more vulnerable for water quality problems, which will reduce fecundity and/or longevity, for example. However, it could be expected that the yield (expressed as average number of nauplii harvested daily) will be several times higher than the optimal yield obtained in a small system. Some variable costs will increase proportionately with the up-scaling; e.g. water costs; feed costs will increase in proportion to the increase of total *Artemia* numbers to be fed. However, energy costs will not increase proportionately with the up-scaling: for running a system 10 times bigger, a proportionate amount of energy will not be needed. Labour will get relatively cheaper: to run a system 10 times bigger, a hatchery owner may need about similar manpower as to run the small system. The fixed costs will also not increase in proportion to the up-scaling; building a system 10 times bigger will not necessarily cost 10 times more: the costs for the air blower, for some of the piping and small devices such as water heaters and feed mixer will remain similar. Therefore, when building and operating the system at alarger scale, it will probably become relatively cheaper and thus the comparison with imported cysts becomes more favourable in favour of the nauplii production system.

7.5. Limitations of the study and recommendations for future research

It is important to recognize the limitations of the present study in relationship to the *Artemia* strains used in the selection of a suitable candidate for culture, and in the practicalities of the water recirculation system and the general operations and maintenance of the system.

The number of *Artemia* strains used was based on their commercial relevance and assumed stress tolerance. However, testing other strains may lead to the validation of more strains as equally suitable or even better candidates for use in low salinity conditions. Such studies might focus on selecting strains such as the Buenos Aires *Artemia persimilis* which have shown adaptation to low salinity (5 – 15 gL^{-1}) (Vanhaecke *et al*., 1984), and obligate ovoviviparous *Artemia* such as the Laysan Lake population in Hawaii, USA which has an assumed genotypical adaptation towards ovoviviparous reproduction (Lenz & Dana, 1987). There is also the population originally from the San Francisco Bay which was introduced in extended coastal saltworks in the area of Rio Grande do Norte, North Eastern Brazil, which seems to have lost its ability to produce cysts (Camara, 2001). This author reported significant cysts production in only six out of 55 saltworks sampled, and concluded that the relatively low incidence of cysts suggests that most of these populations are reproducing ovoviviparously.

Although the water recirculation system (WRS) used for the *Artemia* culture was constructed to enable the harvest of nauplii as they flow along with water from the rearing tanks, the concept was not optimally tested due to time constraints. However, the practicalities for the general operation of the system were easily managed and the system could be up-scaled. Automatic harvest of the nauplii will not only save the labour and time invested in this activity, but will also promote the harvest of instar I nauplii contrary to the mixed harvest of various developmental stages as experienced in this study. This in turn will be more beneficial to the fish larvae as the freshly harvested instar I nauplii are nutritionally considered to be a better and higher-energy food source than the starved instar II (which consume part of their energy). An easy and reliable harvesting procedure could lead to the validation of the system and consequently its acceptance for use in practice. *Artemia* nauplii are positively phototactic (Merchie, 1996). When culture tanks are covered and a light source is positioned in the direction of the outlet fitted with nets of appropriate mesh size (e.g. 500 µm), the animals may swim towards the light, and eventually pass through the mesh net and can be collected in a pouch of smaller mesh size (e.g. 125 µm).

The importance of water quality issues cannot be overemphasized. This was not analyzed during the preliminary work and the laboratory tests in 500 mL bottles. However, the observed poor performance of the animals in some of the treatments (particularly in the bottle tests) indicated that the feed treatment may have affected the quality of the culture water. The absence of data to support this assumption limits the conclusions drawn in this study.

In addition to the low salinity condition for producing nauplii via the ovoviviparous mode in *Artemia,* low-cost equipment and simple processes should be considered in order for the system to be accepted for use in hatcheries. This was the focus of the present study; however, further studies are required before optimal production can be achieved. When the purpose of *Artemia* culture is to produce nauplii, hatchery managers will be more or less stimulated to adopt the technology, based on the availability of the required materials as well as the degree of complexity of the production processes involved. This may include the complexity of the zootechniques, the availability and price of the feed, as well as the difficulty of maintenance and management. Managing of such systems may require the training of personnel on the protocol involved, which is a critical aspect needed for its success and integration in hatcheries.

The feeding regimes used in this study for both the *Artemia* nauplii production system and the fish larval test were sub optimal, due to technical and low nauplii production respectively. This also may have affected the validity of the results. Future studies should be conducted on:

- the optimization of the *Artemia* culture system, for example the duration that such a mass culture system can be maintained without substantial decrease in yield. Focus should be given to the feasibility of keeping a continuous culture within the hatchery by re-starting a

new culture whenever nauplii production begins to drop. Ideally a same culture should run as long as hatchery managers have catfish larvae that are in the first-feeding stage. The phototactic behaviour of the produced nauplii should be explored to enhance harvest of freshly released nauplii. Also, the possibility of applying a more suitable feeding regime using an automatic food distribution apparatus (Dhont and Lavens, 1996) should be considered as it may hold the key to increased nauplii production. This is also applicable for the fish larval culture system where a compressed air feed distributor (Hecht, 2013a) or other types of automatic feed dispensers could be used for the purpose of an optimal delivery method in addition to optimal feeding rates as provided by Verreth and Bieman (1987).

- the dynamics of the stocking density in relation to the performance of the animals. Prolonged studies should be undertaken with focus on monitoring the animals' reproductive period in order to understand and better describe the possible shifts that may occur between ovoviviparity and oviparity; as well as the fluctuations in the ovoviviparous reproduction;
- nutritional aspects such as lipids, carbohydrates, vitamins and energy composition of the ovoviviparous nauplii;
- the issue as to what extent the feed types offered affect the microbial community within the culture media, and consequently the performance of the maternal population in terms of fecundity and type of reproduction, and the nutritional quality of the nauplii. With regard to the shelf life of the feeds, research should be conducted to determine their stability at different storage conditions, such as ambient temperatures, moisture etc.

References

REFERENCES

Aalamifar, H., Agh, N., Malekzadeh, R. & Aalinezhad, M. 2014. A comparative study on the effect of different salinities on the survival, growth, lifespan and morphometric characteristics of cysts of two parthenogenetic species of *Artemia* (GaavKhooni Wetlands of Isfahan, ponds around Lake Urmia) from Iran. *Asian Journal of Biological Science,* 7(6):242-251.

Abatzopoulos, T.J., El-Bermewi, N., Vasdekis, C., Baxevanis, A.D. & Sorgeloos, P. 2003. Effects of salinity and temperature on reproduction and lifespan characteristics of clonal *Artemia*. (International study on Artemia LXVI). *Hydrobiologia,* 492:191-199.

Abatzopoulos, T.J., Baxevanis, A.D., Triantaphyllidis, G.V., Criel, G., Pador, E.L., Van Stappen, G. & Sorgeloos, P. 2006. Quality evaluation of *Artemia urmiana* Günther (Urmia Lake, Iran) with special emphasis on its particular cyst characteristics (International Study on *Artemia,* LXIX). *Aquaculture,* 254:442-454.

Abdoul, B.A. 2016. Promoting agricultural diversification to reduce poverty, fight malnutrition and enhance youth employment opportunities in Eastern Africa. Final report submitted to FAO-Kenya. Project No. GCP/SFE/ 001/MUL. 32p.

Aboua, A.C.D.K. 2017. Overcoming the challenges of fish farming in Africa. Policy brief No. 15, United Nations University – Institute for Natural Resources in Africa UNU – INRA. Available: https://twitter.com/UNUINRA/status/884758064687087616

Abubakar, M.Y., Ipinjolu, J.K., Hassan, W.A. & Alegbeleye, W.O. 2013. Survival and growth of *Clarias gariepinus* (Burchell, 1822) fry on cultured freshwater zooplankton and decapsulated *Artemia. Scientific Journal of Zoology,* 2:54-63.

Adewolu, M.A., Akintola, S.L. & Akinwumi, O.O. 2009. Growth performance and survival of hybrid African catfish larvae (*Clarias gariepinus* X *Heterobranchus birdosalis*) fed on different diets. *The Zoologist,* 7:45-51.

Agh, N., Abatzopoulos, T.J., Van Stappen, G., Razawi Rouhani, S.M. & Sorgeloos, P. 2007. Coexistence of sexual and parthenogenetic *Artemia* populations in Lake Urmia and neighbouring lagoons. *International Review of Hydrobiology,* 92:48-60.

Agh, N., Van Stappen, G., Bossier, P., Sepehri, H., Lofti, V., Razawi Rouhani, S.M. & Sorgeloos, P. 2008. Effects of salinity on survival, growth, reproductive and life span characteristics of *Artemia* populations from Urmia Lake and neighbouring lagoons. *Pakistan Journal of Biological Sciences,* 11(2):164-172.

Akbary, P., Hosseini, S. A., Imanpoor, M., Sudagar, M. & Makhdomi, N. M. 2010. Comparison between live food and artificial diet on survival rate, growth and body chemical composition of *Oncorhynchus mykiss* larvae. *Iranian Journal of Fisheries Sciences*, *9*(1):19–32.

AOAC 2000. Official Methods of Analysis of AOAC international. 16th edition. AOAC 54 International, Gaithersburg, Maryland, USA.

AOAC 2002. Official Methods of Analysis of AOAC international. 17th edition. AOAC 54 International, Gaithersburg, Maryland, USA.

AOAC 2003. Official Methods of Analysis of AOAC international. 19th edition. AOAC 54 International, Gaithersburg, Maryland, USA.

Aragao, C., Conceicao, L.E.C., Dinis, M.T., & Fyhn, H.J. 2004. Amino acid pools of rotifers and *Artemia* under different conditions: Nutritional implications for fish larvae. *Aquaculture,* 234:429-445.

Aruho, C., Ddungu, R., Mwanja, M.T., Wadunde, A.O., Immaculate, K., NKambo, M. & Ondhoro, C.C. 2017. Growth performance and survival of African catfish (*Clarias gariepinus*) fed on different diets. *International Journal of Fisheries and Aquatic Studies*, 5(3):01-05.

Asem, A., Rastegar-Pouyani, N. & Ríos-Escalante, P. 2010. The genus *Artemia* Leach, 1819 (Crustacea : Branchiopoda) I: True and false taxonomical descriptions. *Latin American Journal of Aquatic Research*, 38(3):501–506.

Asem, A. & Eimanifar, A. 2016. Updating historical record on brine shrimp *Artemia* (Crustacea: Anostraca) from Urmia Lake (Iran) in the first half of the 10th century AD. *International Journal of Aquatic Science,* 7(1):3-5.

Asem, A. 2008. Historical record on brine shrimp *Artemia* more than one thousand years ago from Urmia Lake, Iran.*Journal of Biological Research-Thessaloniki*, 9:113–114.

AU-IBAR 2013. Catfish aquaculture industry assessment in Nigeria. Available:www.au-ibar.org.finish.5.1950-catfish-aquaculture-industry-assessment-innigeria

Avnimelech, Y. 1999. Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture,* 179:227-235.

Awaiss, A., & Kestemont, P. 1998. Feeding sequences (rotifer and dry diet), survival, growth and biochemical composition of African catfish, *Clarias gariepinus* Burchell (Pisces: Clariidae), larvae. *Aquaculture Research,* 29:731–741.

Bankole, N.O., Yem, I.Y. & Olowosegun, O.M. 2011. Fish resources of Lake Kainji, Nigeria. In Raji, A., Okaeme, A.N. & Ibeun, M.O. (eds.). Forty years on Lake Kainji Fisheries Research. A publication of National Institute for Freshwater Fisheries Research, New Bussa, Niger State, Nigeria, 20-42.

Barange, M., Merino, G., Blanchard, J.L., Scholtens, J., Harle, J., Allison, E.H., Allen, J.I., Holt, J. & Jennings, S. 2014. Impacts of climate change on marine ecosystem production in societies dependent on fisheries. *Nature climate change*, 4:211-216.

Bardocz, T., Kovacs, E., Radics, F., & Sandor, Zs.S. 1999. Experiments for the improved use of decapsulated *Artemia* cysts in intensive culture of African catfish larvae. *Journal of Fish Biology,* 55:227-232.

Barker-Jorgensen, C. 1966. Feeding, in Edmondson, W.T. (ed.). Marine biology III. Proceedings 3rd International interdisciplinary conference on marine biology. New York Academy of Science, interdisciplinary communications program, New York, USA. 69-133.

Baruah, K., Ranjan, J., Sorgeloos, P. & Bossier, P. 2010. Efficacy of heterologous and homologous heat shock protein 70s as protective agents to *Artemia franciscana* challenged with *Vibrio campbellii. Fish Shellfish Immunology*, 29:733-9.

Baxter-Lowe, L.A., Guo, J.Z., Bergstrom, E.E. & Hokin, L.E. 1989. Molecular cloning of the Na,K-ATPase α-subunit in developing brine shrimp and sequence comparison with higher organisms. *FEBS Letters* 257, 181-187.

Berthelemy-Okazaki, N. & Hedgecock, D. 1987. Effect of environmental factors on cyst formation in the brine shrimp *Artemia*, in Sorgeloos, P., Bengtson, D.A., Decleir, W. & Jaspers, E. (eds.). *Artemia* research and its applications. Vol. 3. Universa Press, Wetteren, Belgium, 167-182.

Bowen, S.T., Buoncristiani, M.R. & Carl, J.R. 1988. *Artemia* habitats: Ion concentrations tolerated by one super species. *Hydrobiologia,* 158:201-214.

Bradley, T.J., 2009. Animal Osmoregulation, Oxford Animal Biology Series. Oxford University Press, Oxford; New York, 168.

Browne, R.A. & Bowen, S.T. 1991. Taxonomy and population genetics of *Artemia*, in Browne, R.A., Sorgeloos, P. & Trotman, C.N.A. (eds.). *Artemia* Biology. C.R.C. Boca Raton, Florida, USA. 221-235.

Browne, R.A. & Wanigasekera, G. 2000. Combined effects of salinity and temperature on survival and reproduction of five species of *Artemia*. *Journal of Experimental Marine Biology and Ecology,* 244(1):29-44.

Busa, W.B. & Crowe, J.H. 1983. Intracellular pH regulates transitions between dormancy and development of brine shrimp (*Artemia salina*) embryos. *Science*, 221:366-368

Cahu, C., Infante, J.Z., & Takeuchi, T. 2003. Nutritional components affecting skeletal development in fish larvae. *Aquaculture,* 227:254-258.

Camara, M.R. 2001. Dispersal of *Artemia franciscana* Kellogg (Crustacea; Anostraca) populations in the coastal saltworks of Rio Grande do Norte, northeastern Brazil. *Hydrobiologia,*466:145–148,

Castro, B.T., Malpica, A.S., Castro, J.M., Castro, G.M. & De Lara, R.A. 2000. Environmental and biological characteristics of *Artemia* ecosystems in México: An updated review, in Munawar, M., Lawrence, S.G., Munawar, I.F. & Malley, D.F. (eds.). Aquatic Ecosystems of México. Status and scope. Backhuys, Leiden, The Netherlands.191-202.

Castro-Mejia, J., Castro-Barrera, T., Hernandez, L.H., Arredondo-Figueroa, J.L., Castro-Mejia, G. & De Lara-Andrade, R. 2011. Effects of salinity on growth and survival in five *Artemia franciscana* (Anostraca: Artemiidae) populations from Mexico pacific coast. *Revista de Biologia Tropical, 59*(1):199-206.

Chan, C.Y., Tran, N., Sulser, T.B., Phillips, M.J., Batka, M., Chimatiro, S. & Dickson, M. 2017. *Fisheries and aquaculture future in Africa*. World Aquaculture Association. Available:https://www.was.org/meetings/_images/MeetingAbstract/WA2017_0583_01.gif.

Chavez, M., Landry, C., Loret, S., Muller, M., Figueroa, J., Peers, B., Rentier-Delrue, F., Krauskopf, M. & Martial, J.A. 1999. APH-1, a POU homeobox gene expressed in the salt gland of the crustacean, *Artemia franciscana. Mechanisms of Development,* 87:207-212.

Chepkirui-Boit, V., Ngugi, C.C., Bowman, J., Oyoo-Okoth, E., Rasowo, J., Mugo-Bundi, J. & Cherop, L. 2011. Growth performance, survival, feed utilization and nutrient utilization of African catfish (*Clarias gariepinus*) larvae co-fed *Artemia* and a micro-diet containing freshwater atyid shrimp (*Caridina nilotica*) during weaning. *Aquaculture Nutrition*, 17:82–89.

Clegg, J.S. & Conte, F.P. 1980. A review of the cellular and developmental biology of *Artemia*, in Persoone, G., Sorgeloos, P., Roels, O. & Jasper E. (eds.). *The Brine Shrimp Artemia*, Vol. 2, Universa Press, Wetteren, Belgium, 11-54.

Clegg, J.S., Willsie, J.K. & Jackson, S.A. 1999. Adaptive significance of a small heat shock/α-crystallin protein (p26) in encysted embryos of the brine shrimp, *Artemia franciscana*. *American Zoologist*, 39:836-847.

Clegg, J.S. & Trotman, C.N.A. 2002. Physiological and biochemical aspects of *Artemia* Ecology, in Abatzopoulos, T.J., Beardmore, J.A., Clegg, J.S. & Sorgeloos, P. (eds.). *Artemia: Basic and Applied Biology.* Netherlands: *Kluwer Academic Publishers.* 129-170.

Cochrane, K., De Youg, C., Soto, D. & Bahri, T. (eds.). 2009. *Climate change implications for fisheries and Aquaculture: Overview of current scientific knowledge.* FAO Fisheries and Aquaculture Technical paper. No 530. Rome, 212.

Connell, C.P.O. & Raymond, L.P. 1970. The effect of food density on survival and growth of early post yolk-sac larvae of the Northern Anchovy (*Engraulismordax Girard*) in the laboratory. *Journal of Experimental Marine Biology and Ecology,* 5:187-197.

Conte, F.P. 1984. Structure and function of the crustacean larval salt gland. *International Review of Cytology,* 91:45-106.

Coutteau, P., Bendonck, L., Lavens, P. & Sorgeloos, P. 1992. The use of manipulated baker's yeast as an algal substitute for the laboratory culture of Anostraca. *Hydrobiologia,* 234:25-32.

Criel, R.J. & Macrae, H.T. 2002. *Artemia* morphology and structure, in Abatzopoulos, T.J., Beardmore, J.A., Clegg, J.S. & Sorgeloos, P. (eds.). *Artemia: Basic and Applied Biology*. Netherlands: Kluwer Academic Publishers. 1–33.

Crowe, J.H., Crowe, L.M., Drinkwater, L & Busa, W.B. 1987. Intracellular pH and anhydrobiosis in

*Artemia*cysts, in Decleir, W., Moens, I., Slegers, H., Sorgeloos P. & Jaspers E. (eds.)., *Artemia Research and its Applications*, Vol. 2, Universa Press, Wetteren, Belgium, 19-40.

Crowe, J.H., Hoekstra, F.A. & Crowe, L.E. 1992. Anhydrobiosis. *Annual Review of Physiology*, 54:579-599.

Crowe, L.M., Reid, D.S. & Crowe, J.H. 1996. Is trehalose special for preserving dry biomaterials? *Biophysical Journal,* 71:2087-2093.

Crowe, J.H., Carpenter, J.F. & Crowe, L.M. 1998. The role of vitrification in anhydrobiosis. *Annual Review of Physiology*, 60:73-103.

Crowe, J.H., Clegg, J.S. & Crowe, L.M. 1998. Anhydrobiosis: the water replacement hypothesis, in Reid, D.S. (ed.). *The Properties of Water in Foods ISOPOW 6*, Chapman and Hall, New York, pp. 440-455.

Dabrowski, K.R. 1986. Ontogenetical aspects of nutritional requirements in fish. *Comp Biochem Physiol. A comp physiol,* 85(4):639-655.

Dattilo, A.M., Bracchini, L., Carlini, L., Loiselle, S. & Rossi, C. 2005. Estimate of the effects of ultraviolet radiation on the mortality of *Artemia franciscana* in naupliar and adult stages*. Int. J Biometeorol*, 49(6):388-395.

Defoirdt, T., Crab, R., Wood, T.K., Sorgeloos, P., Verstraete, W. & Bossier, P. 2006. Quorum sensing-disrupting brominated furanones protect the gnotobiotic brine shrimp *Artemia franciscana* from pathogenic *Vibrio harveyi*, *Vibrio campbellii* and *Vibrio parahaemolyticus* isolates. *Applied and Environmental Microbiology*, 72(9):6419–6423.

Defoirdt, T., Halet, D., Sorgeloos, P., Bossier, P., Verstraete, W. 2006. Short-chain fatty acids protect gnotobiotic *Artemia franciscana* from pathogenic *Vibrio campbellii*. *Aquaculture*, 261(2):804-808.

de Graaf, G. & Janssen, H. 1996. *Artificial reproduction and pond rearing of the African catfish Clarias gariepinus in sub-Saharan Africa* – A Handbook. FAO Fisheries Technical Paper, No. 362. Rome, 73.

De Graaf, J., Amons, R. & Moller, W. 1990. The primary structure of artemin from *Artemia* cysts. *European Journal of Biochemistry*, 193:737-750.

de Moor, I.J., & Bruton, M.N. 1988. *Atlas of alien and translocated indigenous aquatic animals in southern Africa.* A report of the committee for nature conservation research national programme for ecosystem research. South African scientific programmes report No. 144, 310 pp. Pretoria, Foundation for research development, council for scientific and industrial research

De Schryver, P., Crab, R., Defoirdt, T., Boon, N. & Verstraete, W. 2008. The basics of bio-flocs technology: The added value for aquaculture. *Aquaculture,* 277:125–137.

De Vos, S., Van Stappen, G., Sorgeloos, P., Vuylsteke, M., Rombauts, P. & Bossier, P. 2019. Identification of salt stress response genes using the *Artemia* transcriptome. *Aquaculture*, 500:305-315.

De Wet, L. 2000. *Lipids. Aquaculture Nutrition*. Stellenbosch: University of Stellenbosch, South Africa, XI, Matieland 7602. 41.

De Wet, L. 2011. *Aquaculture Nutrition: Protein*. Stellenbosch: University of Stellenbosch, South Africa, XI, Matieland 7602.

Dhont, J. & Lavens, P. 1996. Tank production and use of ongrown *Artemia*, in Lavens, P. & Sorgeloos, P. (eds.). *Manual on the production and use of live food for aquaculture* – FAO fisheries technical paper 361. Rome, FAO. 164-195.

Dhont, J. & Van Stappen, G. 2003. Biology, tank production and nutritional value of *Artemia*, in Støttrup, J.G. & McEvoy, L.A. (eds.). *Live feeds in marine aquaculture*. Blackwell Science Ltd, 9600 Garsington Road, Oxford OX4 2DQ, UK.65-112.

Dobbeleir, J., Adam, N., Bossuyt, E., Bruggeman, E. & Sorgeloos, P. 1980. New aspects of the use of inert diets for high density culturing of brine shrimp, in Persoone, G.P., Sorgeloos, P., Roels, O & Jaspers, E. (eds.). *The brine shrimp Artemia: Ecology, culturing, use in aquaculture, Vol. 3.* Universa Press, Wetteren, Belgium. 165-174.

El-Bermawi, N., Baxevanis, A.D., Abatzopoulos, T.J., Van Stappen, G. & Sorgeloos. P. 2004. Salinity effects on survival, growth and morphometry of four Egyptian *Artemia* populations (International study on *Artemia*, LXVII). *Hydrobiologia,* 523:175-188.

El-Dahhar, A.A., Salama, A.E., El-Greesy, A. & Shaheen, Sh.A. 2013. Effect of live food enrichment and temperature on growth performance survival and digestive tract development of grey mullet, *Liza ramada* larvae. *Journal of the Arabian Aquaculture society,* 8(1):87-104.

El-Magsodi, M. O., Bossier, P., Sorgeloos, P. & Van Stappen, G. 2014. Hatching and nutritional quality of *Artemia* cysts progressively deteriorates as a function of increased exposure to hydration/dehydration cycles. *Aquaculture International*, 22(5):1515–1532.

Emmanuel, O., Chinenye, A., Oluwatobi, A. & Peter, K. 2014. Review of Aquaculture production and management in Nigeria. *American Journal of Experimental Agriculture,* 4:1137-1151.

Engelhard, G.H., Righton, D.A. & Pinnegar, J.K. 2014. Climate change and fishing: A century of fishing distribution in North Sea Cod. *Global change Biology*, 20:2473-2483.

Enyidi, U., Pirhonen, J. & Vielma, J. 2014. Effects of substituting soybean (*Glycine max*) meal with bambaranut (*Voandzeia subterranea*) meal on growth performance and survival of African catfish (*Clarias gariepinus*) larvae. *International Journal of Fisheries and Aquatic Studies*, 1(3):152–157.

Evjemo, J. O. & Olsen, Y. 1999. Effect of food concentration on the growth and production rate of *Artemia franciscana* feeding on algae (T-iso). *Journal of Experimental Marine Biology and Ecology*, 242(2):273–296.

Fakoya, K.A., Owodeinde, F.G., Jimoh, A.A. & Akintola, S.L. 2005. An overview of the challenges and prospects in developing an aquaculture industry in Lagos state, Nigeria. Available: https://www.researchgate.net/publication/277195568.

FAO 2003. Food energy – methods of analysis and conversion factors. Food and nutrition paper 77. Report of a technical workshop. FAO, Rome, 93p.

FAO 2010. State of the world fisheries and aquaculture, FAO, Rome, 218p.

FAO 2011. Cultured aquatic species information programme. *Artemia* spp. in, *FAO Fisheries and Aquaculture Department*, Rome. Available: http://www.fao.org/fishery/culturedspecies/Artemia_spp/en.

FAO 2016. The state of world fisheries and aquaculture: Contribution for food security and nutrition for all. FAO, Rome, 204p.

FAO 2017a. FAO Fisheries and Aquaculture Information and Statistic branch. Available: http://www.fao.org/fishery/ statistics/en.

FAO 2017b. FAO Country programming framework (CPF). Federal Republic of Nigeria. Available:www.fao.org/3/a-auo53e.pdf.

FAO 2018. The State of world fisheries and aquaculture 2018 – Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO. 227p.

FDF 2017. Fisheries statistics of Nigeria. Available: www.nan.ng

Fiksen, O. & Jørgensen, C. 2011. Model of optimal behaviour in fish larvae predicts that food availability determines survival, but not growth. *Marine Ecology Progress Series*, 432:207–219.

Folch, J., Lees, M. & Sloane-Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226:495-509.

Gajardo, G.M. & Beardmore, J.A. 2012. The brine shrimp *Artemia*: Adapted to critical life conditions. *Frontiers in Physiology,* 3(185):1-8.

Garcia-Ortega, A., Verreth, J. A. J., Coutteau, P., Segner, H., Huisman, E. A. & Sorgeloos, P. 1998. Biochemical and enzymatic characterization of decapsulated cysts and nauplii of the brine shrimp *Artemia* at different developmental stages. *Aquaculture*, 161(1–4):501–514.

Garcia-Ortega, A., Verreth, J., Van Hoornyck, A. & Segner, H. 2000. Heat treatment affects protein quality and protease activity in decapsulated cysts of *Artemia* when used as starter food for larval of African catfish *Clarias gariepinus* (Burchell). *Aquaculture Nutrition,* 6:25-31.

García-Ortega, A., Verreth, J., Vermis, K., Nelis, H., Sorgeloos, P. & Verstegen, M. 2008. Laboratory investigation of daily food intake and gut evacuation in larvae of African catfish *Clarias gariepinus* under different feeding conditions. *Aquaculture International,* 18:119-134.

Garcia, S. M. & Newton, C. 1995. Current situation, trends and prospects in world capture fisheries. Paper presented at the conference on fisheries management. Global trends. Seattle (Washington, USA), 14-16 June 1994 FAO, 63p. Available: http://horizon.documentation.ird.fr/exl-doc/pleins_textes/pleins_textes_6/b_fdi_37-38/43004.pdf.

Garling, D.L. Jr. & Wilson, R.P. 1976. Optimum dietary protein to energy ratio for channel catfish fingerlings, *Ictalurus punctatus*. *Journal of Nutrition,* 106:1368-1375.

Gopakumar, D. 2009. Current status of live food production for larviculture. *CMFRI - Winter School Course Manual on "Recent Advances in Breeding and Larviculture of Marine Finfish and Shellfish",* 56-60.

Hamre, K., Yufera, M., Roonestad, I., Boglione, C., Concecao, L.E.C. & Izquierdo, M. 2013. Fish larval nutrition and feed formulation: Knowledge gaps and bottlenecks for advances in larval rearing. *Reviews in Aquaculture.* 5(1):526-558.

Hasan, M.R. 2016. *Status of world aquaculture and global aqua feed requirement with special notes on Artemia.* Presentation at the FAO expert workshop on sustainable use and management of *Artemia* resources in Asia. Tianjin, China, 7-9 November 2016. Available: http://www.fao.org/documents/card/en/ [4-24].

Haylor, G.S. 1993. Controlled hatchery production of African catfish, *Clarias gariepinus* (Burchell): an overview. *Aquaculture and Fisheries Management,* 24:245–252.

Hebrew University of Jerusalem. 2017. New innovation feeds the world with more fish protein. *Science Daily*. Available: www.sciencedaily.com/releases/2017/06/170627141851.htm

Hecht, T. & Appelbaum, S. 1987. Notes on the growth of Israeli sharptooth catfish (*Clarias gariepinus*) during the primary nursing phase. *Aquaculture*, 63:195–204.

Hecht, T. 1996. An alternative life history approach to the nutrition and feeding of *Siluroidei* larvae and early juveniles. *Aquatic Living Resources,* 9:121–133.

Hecht, T. 2013a. A review of on-farm feed management practices for North African catfish (*Clarias gariepinus*) in sub-Saharan Africa, in Hasan, M.R. & New, M.B. (eds.). *On-farm feeding and feed management in aquaculture*. FAO Fisheries and Aquaculture Technical paper No. 583. FAO, Rome. 463-479.

Hecht, T. 2013b. Species profile: North African catfish [*Clarias gariepinus* (Burchell, 1822)], in Aquaculture feed and fertilizer resources information system. FAO, Rome. Available: www.fao.org/fishery/affris/en/.

Hedayati, A. & Bagheri, T. 2010. Effect of some physico-chemical parameters on hatching ability in cyst of *Artemia* urmania. *World Journal of Zoology,* 5(4):295-297.

Heimann, W. 1980. Fundamentals of food chemistry. 91.

Hoa, N.V., Anh, N.T.N., Ngan, P.T.T., Toi, H.T. & Le, T.H. 2007. *Artemia*-Research and application in aquaculture. Technical book. Can Tho University, Agricultural publisher, Vietnam, 17-23.

Hogendoorn, H. 1980. Controlled propagation of the African catfish, *Clarias lazera* (C. and V.). III: feeding and growth of fry. *Aquaculture,* 21:233–241.

Holliday, C.W., Roye, D.B. & Roer, R.D. 1990. Salinity-induced changes in branchial Na^+/K^+-ATPase activity and transepithelial potential difference in the brine shrimp, *Artemia salina. Journal of Experimental Biology*, 151:279-296.

Interaminense, J.A., Calazans, N.F., DoValle, B.C., Vogeley, J.L., Peixoto, S. & Soares, R. 2014. Vibrio spp control at brine shrimp, *Artemia*, hatching and enrichment. *Journal of the World Aquaculture Society,* 45:65-74.

Intriago, P. & Jones, D.A. 1993. Bacteria as food for *Artemia. Aquaculture,* 113:115-127.

Jackson, P.E. 2000. Ion Chromatography in Environmental Analysis, in Meyers, R.A. (ed.). *Encyclopedia of Analytical Chemistry*. John Wiley & Sons Ltd, Chichester. 2779-2801.

Kadhar, A., Kumar, A., Ali, J. & John, A. 2014. Studies on the survival and growth of fry of Catla catla (Hamilton, 1922) using live feed. *Journal of marine biology,*2014:7.

Kaiser, H., Gordon, A.K. & Paulet, T.G. 2006. Review of the African distribution of the brine shrimp genus *Artemia*. *Water SA*, 32:597-603.

Kamaraj, K., Kathiravan, C. & Jayakumar, A. 2014. Entrepreneurship – A possible solution to the surplus population, *Journal of Business Management and Social Science Research,* 3(1):27–32.

Kanazawa, A., Teshima, S.T., Sakamoto, M. & Awal, M.A. 1980. Requirement of *Tilapia zillii* for essential fatty acids. *Bulletin of the Japanese Society of Scientific Fisheries,* 46(11):1353-1356.

Kappas, I., Abatzopoulos, T.J., Van Hoa, N., Sorgeloos, P. & Beardmore, J.A. 2004. Genetic andreproductive differentiation of *Artemia franciscana* in a new environment. *Marine Biology*, 146:103–117.

Kerdchuen, N. & Legendre, M. 1994. Larval rearing of an African catfish, *Heterobranchus longfilis*, (Teleostei, Clariidae): a comparison between natural and artificial diet. *Aquatic Living Resources*, 7:247–253.

Kulasekarapadian, S. & Ravichandran, P. 2003. *Artemia* cysts production at Kelambakkam near Chennai. *J mar boil Ass India,* 45:166-177.

Lavens, P. & Sorgeloos, P. 1984. Controlled production of *Artemia* cysts under standard conditions in a recirculating culture system. *Aquaculture Engineering,* 3:221-235.

Lavens, P., Baert, P., De Meulemeester, A., Van Ballaer, E. & Sorgeloos, P. 1985. New developments in the high density flow-through culturing of brine shrimp *Artemia. J. World Aquaculture Society,* 16:498-508

Lavens, P. & Sorgeloos, P. 1987. Design, operation and potential of a culture system for the continuous production of *Artemia* nauplii. *Artemia Research and its application,* in Sorgeloos, P., Bengtson, D.A., Decleir, W. & Jasper, E. (eds.). *Ecology, culturing, use in aquaculture. Universa press, Wetteren, Belgium,* 3:340-345.

Lavens, P., De Meulemeester, A. & Sorgeloos, P. 1987. Evaluation of mono and mixed diets as food for intensive *Artemia* culture. *Artemia Research and its application,* in Sorgeloos, P., Bengtson, D.A., Decleir, W. & Jasper, E. (eds.). *Ecology, culturing, use in aquaculture. Universa press, Wetteren, Belgium,* 3:309-318.

Lavens, P. & Sorgeloos, P. 1991. Production of *Artemia* in culture tanks, in Browne R.A., Sorgeloos, P. & Trotman, C.N.A. (eds.). *Artemia* Biology, 317-349.

Lavens, P. & Sorgeloos, P. (eds.). 1996. Manual on the production and use of live food for aquaculture. *FAO fisheries technical paper 361.*FAO, Rome.

Lavens, P. & Sorgeloos, P. 2000. The history, present status and prospects of the availability of *Artemia* cysts for aquaculture. *Aquaculture*, *181*(3-4):397–403.

Lee, K.J. & Watts, S.A. 1994. Specific activity of Na^+ K^+ ATPase is not altered in response to changing salinities during early development of the brine shrimp, *Artemia franciscana*. *Physiological Zoology*, 67:910-924.

Lenz, P. & Dana, G. 1987. Life-cycle studies in *Artemia*: a comparison between a subtropical and a temperate population, in Sorgeloos, P., Bengtson, D.A., Decleir, W. & Jasper, E. (eds.). *Artemia research and its application. Universa press, Wetteren, Belgium.* 88-100.

Le Ruyet, J. P., Alexandre, J. C., Thébaud, L. & Mugnier, C. 2007. Marine fish larvae feeding: Formulated diets or live prey? *Journal of the World Aquaculture Society*, 24:211-224.

Liang, P. & Macrae, T.H. 1999. The synthesis of a small heat shock/α-crystallin protein in *Artemia* and its relationship to stress tolerance during development. *Developmental Biology*, 207:445-456.

Lim, L.C., Cho, Y.L., Dhert, P., Wong, C.C., Nelis, H., & Sorgeloos, P. 2002. Use of decapsulated *Artemia* cysts in ornamental fish culture. *Aquaculture Research,* 33:575-589.

Litvinenko, L.I., Litvinenko, A.I., Boiko, E.G. & Kutsanov, K. 2015. *Artemia* cyst production in Russia. *Chinese Journal of Oceanology and Limnology*, 33:1436-1450.

Maldonado-Montiel, T. D. N. J., Rodriguez-Canche, L. G. & Olvera-Novoa, M. A. 2003. Evaluation of *Artemia* biomass production in San Crisanto, Yucatan, Mexico, with the use of poultry manure as organic fertilizer. *Aquaculture*, *219*(1-4):573–584.

Martin, E & Hine, R.S. 2000. Protein synthesis. in Martin, E.& Hine, R.S. (eds.). Dictionary of Biology. Fourth Edition, Oxford University press, Great Clarendon street, Oxford OX2 6DP. 489.

Marques, A., Francois, J.M., Dhont, J., Bossier, P. & Sorgeloos, P. 2004. Influence of yeast quality on performance of gnotobiotically grown *Artemia*. *J Exp Mar BiolEcol*, 310:247-264.

Matthew, M., Justus, R., Constantine, O., Richard, D. & Casius, A. 2015. Current fish practices in Uganda: The potential for future investment. *International Journal of Fisheries and Aquatic Studies,* 2(4):224-232.

Mazid, M. A., Tanaka, Y., Katayama, T., Rahman, M.A., Simpson, K.L. & Chichester, C.O. 1979. Growth response of *Tilapia zillii* fingerlings fed isocaloric diets with variable protein levels. *Aquaculture,*18:115-122.

Megahed, M.E. & Aly, S.M. 2009. Challenges facing marine aquaculture and requirements for development in Egypt. Abbassa*International Journal for Aquaculture*. 505-527. Available: https://www.reserachgate.net/publication/279974065

Merchie, G. 1996. Use of nauplii and metanauplii, in Lavens, P. & Sorgeloos, P (eds.). *Manual on the production and use of live food for Aquaculture. FAO Fisheries Technical Paper No. 361. Rome, FAO. 137-163.*

Mukai, Y., Tuzan, A.D., Lim, L.S., Wahid, N., Sitti Raehanah, M.S. & Senoo, S. 2008. Development of sensory organs in larvae of African catfish *Clarias gariepinus*. *Journal of Fish Biology*, 73:1648-1661.

Mukai, Y. & Seng Lim, L. 2011. Larval rearing and feeding behaviour of African catfish *Clarias gariepinus* under dark conditions. *Journal of Fisheries and aquaculture science,* 6:272-278.

Munguti, J.M. & Ogello, E.O. 2014. An overview of Kenyan aquaculture: current status, challenges and opportunities for future development. *Fish. Aquat. Sci.,* 17:1–11.

Naegel, L.C.A. 1999. Controlled production of *Artemia* biomass using an inert commercial diet compared with microalgae Chaeteceros. *Journal of Aquaculture Engineering,* 21:49-59.

National Bureau of Statistics (2017). Nigeria's fish production: 2010 – 2015. A report of the National Bureau of Statistics (NBS). 1-20.

Ndavano, K. 2012. *Making billionaires out of small time fishing*. Available: http://www.kmfri.co.ke/artemia.html .

NEPAD, 2013. Planning and coordinating agency. Aquaculture a technical body of the African Union. Available: www.nepad.ord/foodsecurity/fisheries/aquaculture.

Nose, T. 1979. Summary report on the requirements of essential amino acids for carp, in Tiews, K. & Halver, J.E. (eds.). Finfish nutrition and fish feed technology, Berlin: Heenemann GmbH. 145-156.

NRC. 1993. Nutrient requirements of fish. National Academy Press, 2101, constitution avenue, NW, Washington, D.C. 20418. P124. Availble: http://www.nap.edu/catalog/2115.html.

Nunes, B.S., Carvalho, F.D., Guilhermino, L.M. & Van Stappen, G. 2006. Use of the genus *Artemia* in ecotoxicology testing. *Environmental pollution,* 144:453-462.

Ogello, E.O., Kembenya, E., Githukiya, C.M., Nyonje, B.M. & Munguti, J.M. 2014.The occurrence of the brine shrimp, *Artemia franciscana* (Kellog 1906) in Kenya and the potential economic impacts among Kenyan coastal communities. *International Journal of Fisheries and Aquatic Studies,* 1(5):151-156.

Ogino, C. & Saito, K. 1970. Protein nutrition in fish. 1. The utilization of dietary protein by young carp. *Bulletin of the Japanese Society of Scientific Fisheries,* 36:250-254.

Olaniyi, W.A. &Omitogun, O.G. 2013. Stages in the early and larval development of the African catfish *Clarias gariepinus* (Teleostei, Clariidae). *Zygote,* 17p.

Olokor, J.O. 2011. Geography and climate of Kainji Lake Basin, in Raji, A., Okaeme, A.N. & Ibeun, M.O. (eds.). Forty years on Lake Kainji Fisheries Research. A publication of National Institute for Freshwater Fisheries Research, New Bussa, Niger State, Nigeria, 11-19.

Olson, R. R. & Olson, M. H. 1989. Food limitation of planktotrophic marine invertebrate larvae: Does it control recruitment success? *Annual Review of Ecology and Systematics*, *20*(1):225–247.

Onura, C.N., Broeck, W.N., Nevejan, N., Muendo, P. & Van Stappen, G. 2018. Growth performance and intestinal morphology of African catfish (*Clarias gariepinus*, Burchell, 1822) larvae fed on live and dry feeds. *Aquaculture*, 489:70-79,

Osman, A.G.M., Wuertz, S., Mekkawy, I.A., Verreth, J. & Kirschbaum, F. 2008. Early development of the African catfish *Clarias gariepinus* (Burchell, 1822), focusing on the ontogeny of selected organs. *Journal of Applied Ichthyology,* 24:187–195.

Ovie, S.I. 1997. The Ecology and Culture of the Zooplankton of Jebba Lake, Nigeria.Doctoral dissertation, University of Ilorin, Nigeria. 240p.

Ownagh, E., Agh, N. & Noori, F. 2015. Comparison of the growth, survival and nutritional value of *Artemia* using various agricultural by-products and unicellular algae *Dunaliella salina. Iranian Journal of Fisheries science*. 14(2):358-368.

Pablo, I. & Jones, D.A. 1993. Bacteria as food for *Artemia*. *Aquaculture*, 113:115-127.

Paray, B.A., Haniffa, M.A., Innocent, X. & Rather, A. 2016. Effect of feed quality on growth and survival of stripped snakehead, *Channa striatus* (Bloch, 1793) hatchlings. *Indian Journal of Geo-Marine Science,* 45:105-110.

Persoone, G. & Sorgeloos, P. 1980. General aspects of the ecology and biogeography of *Artemia*, in Persoone, G., Sorgeloos, P., Roels, O., Jaspers, E. (eds.). *The brine shrimp Artemia*, Vol. 3. Wetteren, Belgium. Universa Press, 3-24.

Pinto, P.M., Almeida, V.D. & Vieira, N. 2012. Combined effects of salinity and temperature on survival of two autochthonous parthenogenetic populations of *Artemia* (Crustacea: Anostraca) in Portugal (Rio Maior and Aveiro saltworks). *International Journal of Artemia Biology,* 2(2):43-47.

Pomeranz, Y. & Meloan, C.E. 1971. Food Analysis: Theory and practice. 578-603.

Post, F.J. & Youssef, N.N. 1977. A prokaryotic intracellular symbiont of the Great Salt Lake brine shrimp *Artemia* salina (L.). *Canadian Journal of Microbiology,* 23:1232-1236.

Potongkam, K.& Miller, J. 2006. *Manual on catfish hatchery and production. A guide for small to medium scale hatchery and farm producers in Nigeria.* Aquaculture and inland fisheries project. FAO, National special programme for food security (NSPFS). 42p. Available: http://www.fao.org/3/a-ak505e.pdf.

Provasoli, L. & Shiraishi, K. 1959. Axenic cultivation of the brine shrimp *Artemia* salina. *Biol. Bull*., 1 17:347-355.

Qi, Z. 2016. *Microbiome: Criteria for the quality control of Artemia product.* Presentation at the FAO expert workshop on sustainable use and management of *Artemia* resources in Asia. Tianjin, China, 7-9 November 2016. Available: http://www.fao.org/documents/card/en/ [282-393].

Rice, M.A., Bengtson, D.A.& Jaworski, C. 1994. *Evaluation of artificial diets for cultured fish.* Northeastern Regional Aquaculture Centre, University of Massachusetts, Dartmouth North, Dartmouth Massachusetts 02747, NRAC Fact Sheet No.222, 4p.

Rice, J. C. & Garcia, S. M. 2011. Fisheries, food security, climate change, and biodiversity: characteristics of the sector and perspectives on emerging issues. *ICES Journal of Marine Science: Journal Du Conseil*, 68(6):1343–1353.

Royan, J.P., Vijayaraghavan, S. & Krishna Kumari, L. 1990. Assessment of cysts production potential of a natural population of brine shrimp *Artemia*.*Indian Journal of Marine Sciences*, 20:72-74.

Rubio, V.C., Sanchez, F.J. & Madrid, J.A. 2003. Macronutrient selection through post ingestive signals in sea bass fed on gelatine capsules. *Physiology and Behavior,* 78:795-803.

Ruiz, O., Amat, F. & Navarro, J.C. 2008. A comparative study of the fatty acid profile of *Artemia franciscana* and *A. persimilis* cultured at mesocosm scale. *Journal of Experimental Marine Biology and Ecology* 354:9-16.

Sales, J. 2011. First feeding of freshwater fish larvae with live feed versus compound diets: A meta-analysis. *Aquaculture International,* 19:1217-1228.

Sanchez-Vazquez, F.J., Yamamoto, T., Akiyama, T., Madrid, T.A. & Tabata, M. 1999. Macronutrient self-selection through demand-feeders in rainbow trout. *Physiology and Behavior.* 66:45-51.

Sanni, A.O. 2018. Factors influencing the adoption of improved aquaculture technologies by fish farmers in Kainji and Jebba Lake Basins area, Nigeria. Doctoral dissertation, Federal University of Technology, Minna, Niger State, Nigeria.

Santiago, C.B.& Lovell, R.T. 1988. Amino acid requirements for growth of Nile tilapia. *Journal of Nutrition,* 118:1540-1546.

Satia, B. 2010. Aquaculture development in Africa: Current status and future prospects, in Network of Aquaculture Centres in Asia-pacific NACA. Available: www.enaca.org/modules.

Satoh, S., Poe, W.E. & Wilson, R.P. 1989. Effect of dietary n-3 fatty acids on weight gain and liver polar lipid fatty acid composition of fingerling channel catfish. *Journal of Nutrition,* 119: 23-28.

Schoo, K.L., Aberle, N., Malzahn, A.M., Schmalenbach, I. & Boersma, M. 2014. The reaction of European lobster larvae (*Homarus gammarus*) to different quality food: Effects of ontogenetic shifts and pre-feeding history. *Oecologia*, *174*(2):581–594.

Singh, R.K., & Khandagale, P.A. 2006. Optimal water characteristics for commercial production of cysts of the brine shrimp, *Artemia* in salt ponds. *Crustaceana*, 79(8):913-923.

Soniraj, N. 2004. Effect of salinity on the life span and reproductive characteristics of brine shrimps in the salt pans in Tuticorin. *Journal of the Marine Biological Association of India,* 46(2):133-140.

Sorgeloos, P., Baeza-Mesa, M., Bossuyt, E., Bruggeman, E., Dobbeleir, J., Vaersichele, D., Lavina, E. & Bernardino, A. 1980. Culture of *Artemia* on rice bran: The conversion of a waste-product into highly nutritive animal protein. *Aquaculture,* 21:393-396.

Sorgeloos, P., Lavens, P., Le´ger, P., Tackaert, W. &Versichele, D. 1986. Manual for the culture and use of the brine shrimp *Artemia* in aquaculture. Faculty of Agriculture, State University of Ghent, Belgium.

Sorgeloos, P., Dhert, P. & Candreva, P. 2001. Use of the brine shrimp, *Artemia* spp., in marine fish larviculture. *Aquaculture*, 200(1–2):147–159.

Sorgeloos, P. 2016. *Forty years of research and use of brine shrimp Artemia spp*. Presentation at the FAO expert workshop on sustainable use and management of *Artemia* resources in Asia. Tianjin, China, 7-9 November, 2016. Available: http://www.fao.org/documents/card/en/ [25-49].

Soundarapandian, P. & Saravanakumar, G. 2009. Effect of different salinities on the survival and growth of *Artemia* spp, *Current Research Journal of Biological Sciences,* 1(2):20–22.

Stottrup, J. & McEvoy, L. (eds.). 2002. *Live feeds in marine aquaculture*. Blackwell Science Ltd, 9600 Garsington Road, Oxford OX4 2DQ, UK.

Sui, L. & Xin, N. 2016. *History and current status of Artemia research and applications in Bohai Bay Area. Presentation at the FAO expert workshop on sustainable use and management of Artemia resources in Asia*. Tianjin, China, 7-9 November, 2016. Available:http://www.fao.org/documents/card/en/

Sung, Y.Y., Pineda, C., MacRae, T.H., Sorgeloos, P., Bossier, P., 2008. Exposure of gnotobiotic *Artemia franciscana* larvae to abiotic stress promotes heat shock protein 70 synthesis and enhances resistance to pathogenic *Vibrio campbellii*. *Cell Stress Chaperones,* 13:59–66.

Tacon, A.G.J. 2010. Aquaculture: A catch for all? OECD Observer No 278:32. Available:http://oecdobserver.org/news/archivestory.php/aid/3230/Aquaculture:_A_catch_for_all_.html

Takeuchi, T. & Watanabe, T. 1977. Requirement of carp for essential fatty acids. *Bulletin of the Japanese Society of Scientific Fisheries,* 43:541-551.

Takeuchi, T., Watanabe, T. & Ogino, C. 1979. Optimum ratio of dietary energy to protein for carp. *Bulletin of the Japanese Society of Scientific Fisheries,* 45:983-987.

Takeuchi, T., Satoh, S. & Watanabe, T. 1983. Requirement of *Tilapia nilotica* for essential fatty acids. *Bulletin of the Japanese Society of Scientific Fisheries,* 49:1127-1134.

Talloen, M. 1978. The use of locally available food for the mass culture of the brine shrimp *Artemia salina. Bull. Japara Brackish water Aquaculture Development Center.*

Teugels, G.G. 1984. The nomenclature of African *Clarias* species used in aquaculture. *Aquaculture,* 38:373-374.

Tidwell, J.H. 2012. *Aquaculture Production Systems*. *World aquaculture society, Wiley-Blackwell – John Wiley and sons, Ltd publication*. Available: http://doi.org/10.1002/9781118250105.

Toi, H.T., Boeckx, P., Sorgeloos, P., Bossier, P. & Van Stappen, G. 2013. Bacteria contribute to *Artemia* nutrition in algae-limited conditions: A laboratory study. *Aquaculture,* 388–391:1–7.

Treece, G. D. & Davis, D. A. 2000. Culture of small zooplankters for the feeding of larval fish. *Southern Regional Aquaculture Center*, *SRAC publication No* 701 Available: https://www.ncrac.org/files/biblio/SRAC0701.pdf

Triantaphyllidis, G.V., Poulopoulou, K., Abatzopoulos, T., Perez, C.A.P. &Sorgeloos, P. 1995. International study on *Artemia* XLIX. Salinity effect on the survival, maturity, growth, biometrics reproductive and lifespan characteristics of a bisexual and parthenogenetic population of *Artemia*. *Hydrobiologia,* 302:215-225.

Triantaphyllidis, G.V., Criel, G.R.J., Abatzopoulos, T.J. & Sorgeloos. P. 1997a. International study on *Artemia*. LIII. Morphological study of *Artemia* with emphasis to Old World populations. I. Bisexual populations. *Hydrobiologia,* 357:139-153.

Triantaphyllidis, G.V., Criel, G.R.J., Abatzopoulos, T.J., Thomas, K.M., Peleman, J., Beardmore, J.A. & Sorgeloos, P. 1997b. International study on *Artemia*. LVII. Morphological and molecular characters suggest conspecificity of all bisexual European and North African *Artemia* populations. *Marine Biology*. 129:477-487.

Triantaphyllidis, G.V., Abatzopoulos, T.J. & Sorgeloos, P. 1998. Review of the biogeography of the genus *Artemia* (Crustacea, Anostraca). *Journal of Biogeography*. 25:213-226.

United Nations, 2015. Department of economics and social affairs. Available: www.un.org/en/development/desa/news/populations/2015-report.html

Uys, W.& Hecht, T. 1985. Evaluation and preparation of a suitable dry feed and optimal feeding frequency for the primary nursing of *Clarias gariepinus* larvae (Pisces: Clariidae). *Aquaculture,* 47:173–183.

Vadstein, O., Attramadal, K.J.K., Bakke, I.&Olsen, Y. 2018.K-Selection as microbial community management strategy: A method for improved viability of larvae in Aquaculture. *Frontiers in Microbiology,* 9(2730):1-17.

Vanhaecke, P. & Sorgeloos, P. 1980. International study on *Artemia.* XVI. Growth and survival of *Artemia* larvae of different geographical origin in a standard culture test. *Mar. Ecol. Prog. Ser.,* 3:303-307.

Vanhaecke, P. & Sorgeloos, P. 1980. International study on Artemia IV. The biometrics of Artemia strains from different geographical origin, in Persoone, G., Sorgeloos, P., Roels, O. & Jaspers, E. (eds.). The brine shrimp Artemia Vol. 3. Ecology, culturing, use in Aquaculture. Universa press, Watteren, Belgium. 393-405.

Vanhaecke, P., Siddall, S.E. & Sorgeloos, P. 1984. International study on *Artemia*. XXXII. Combined effects of temperature and salinity on the survival of *Artemia* of various geographical origins. *Journal of Experimental Marine Biology and Ecology,* 80:259-275.

Vanhaecke, P., Tackaert. W. & Sorgeloos, P. 1987. The biogeography of *Artemia*: an updated review, in Sorgeloos, P., Bengtson, D.A., Decleir, W. & Jaspers, E. (eds.). *Artemia Research and its Applications,* Vol. 1. Wetteren, Belgium. Universa Press, 129-155.

Van, N.T.H., Van Hoa, N., Bossier, P., Sorgeloos, P.& Van Stappen, G. 2014. Mass selection for small-sized cysts in *Artemia franciscana* produced in Vinh Chau salt ponds, Vietnam. *Aquaculture Research*, *45*(10):1591–1599.

Van Stappen, G. 1996a. Use of cysts, in Lavens, P. & Sorgeloos, P. (eds.). *Manual on the production and use of live food for Aquaculture.FAO Fisheries Technical Paper No. 361. Rome, FAO.*107-130.

Van Stappen, G. 1996b. Introduction, biology and ecology of *Artemia,* in Lavens, P. & Sorgeloos, P. (eds.). *Manual on the production and use of live food for Aquaculture.FAO Fisheries Technical Paper No. 361. Rome, FAO.*79-106.

Van Stappen, G. 2002. Zoogeography, in Abatzopoulos, T.J., Beardmore, J.A., Clegg, J.S. &Sorgeloos, P. (eds.). Artemia *Basic and Applied Biology*. Netherlands: Kluwer Academic Publishers, 171-215.

Van Stappen, G. 2008. *Artemia* biodiversity in Central and Eastern Asia. Doctoral dissertation, Ghent University, Belgium.

Van Stappen, G. 2011. Cultured aquatic species information programme. *Artemia spp*, in *FAO Fisheries and Aquaculture Department. Rome*.
Available: http://www.fao.org/fishery/culturedspecies/Artemia.spp/en.

Vasudevan, S. 2012. A biometrical, morphological and biochemical characterization of three *Artemia* (crustacean, Anostraca) populations from south India. *International Journal of Artemia biology,* 2(2):7-29.

Verdonck, L., Grisez, L., Sweetman, E., Minkoff, G., Sorgeloos, P., Ollevier, F. & Swings, J. 1994. Variability of the microbial environment of rotifer, *Brachionus plicatilis* and *Artemia* production systems. *Journal of World Aquaculture Society,* 25:55-59.

Verreth, J., & Bieman, D. 1987. Quantitative feed requirements of African catfish (*Clarias gariepinus*) Burchell larvae fed with decapsulated cysts of *Artemia* I. The effect of temperature and feeding level. *Aquaculture,* 63:251-267.

Verreth, J., Storch, V. & Segner, H. 1987. A comparative study on the nutritional quality of decapsulated *Artemia* cysts, micro encapsulated egg diets and enriched dry feeds for *Clarias gariepinus* (Burchell) larvae. *Aquaculture,* 63:269-282.

Verreth, J. & Van Tongeren, M. 1989. Weaning time in *Clarias gariepinus* (Burchell) larvae. *Aquaculture,* 83:81-88.

Verreth, J., Eding, E.H., Rao, G.R.M., Husken, F. & Segner, H. 1993. A review of feeding practices, growth and nutritional physiology on larvae of the catfishes *Clarias gariepinus* and *Clarias batrachus*. *Journal of the World Aquaculture Society,* 24:135-144.

Verreth, J. 1994. Nutrition and related ontogenetic aspects in larvae of the African catfish, *Clarias gariepinus*. Doctoral dissertation.

Versichele, D. & Sorgeloos, P.1980. Controlled production of *Artemia* cysts in batch cultures, in Persoone, G., Sorgeloos, P., Roels, O. & Jasper, E. (eds.). *The brine shrimp Artemia vol. 3. Ecology, culture, use in aquaculture*. Universa press, Wetteren, Belgium.231-246.

Wang, K. W., Takeuchi, T. & Watanabe, T. 1985. Effect of dietary protein levels on growth of *Tilapia nilotica*. *Bulletin of the Japanese Society of Scientific Fisheries,* 51:133-140.

Warner, A.H. 1992. Diguanosine and related nonadenylated polyphosphates, in McLennan, A.G. (ed.)., *Ap_4A and other Dinucleoside Polyphosphates*, CRC Press, Boca Raton, Florida, pp. 275-303.

Watanabe, T., Takeuchi, T. & Ogino, O. 1975. Effect of dietary methyl linoleate and linolenate on growth of carp-2. *Bulletin of the Japanese Society of Scientific Fisheries,* 41:263-269.

Watanabe, T., Kitajima, C. & Fujita, S. 1983. Nutritional values of live organisms used in Japan for mass propagation of fish: A review. *Aquaculture*, *34*(1–2):115–143.

Watts, S.A., Yeh, E.W. & Henry, R.P. 1996. Hypoosmotic stimulation of ornithine decarboxylase activity in the brine shrimp *Artemia franciscana*. *Journal of Experimental Zoology*, 274:15-22.

Wear, R.G. & Haslett, S.J. 1986. Effects of temperature and salinity on the biology of *Artemia franciscana* (Kellogg) from Lake Grasmere New Zealand. 1. Growth and mortality. *Journal of Experimental Marine Biology and Ecology,* 98:153-166.

Wear, R.G., Haslett, S.J. & Alexander, N.K. 1986. Effects of temperature and salinity on the biology of *Artemia franciscana* (Kellogg) from Lake Grasmere, New Zealand 2. Maturation, fecundity and generation times. *Journal of Experimental Marine Biology and Ecology,* 98:167-183.

Wedemeyer, G.A. 1996. Physiology of fish in intensive culture systems. Chapman and Hall, 115 Fifth Avenue, New York, NY 10003. 225p.

Wilson, R.P., Allen, O.W. Jr., Robinson, E.H. & Poe, W.E. 1978. Tryptophan and threonine requirements of fingerling channel catfish. *Journal of Nutrition,* 108:1595-1599.

Wing-Keong, Ng. 2017.*Clarias gariepinus: Invasive species compendium.* Available: http://www.cabi.org/isc/datasheet/88683

Yilmaz, E. 2005. Effect of two chemo-attractants and different first feeds on the growth of African catfish (*C. gariepinus*, Burchell, 1822) at different Laval stages. *Turk. J. Vet. Anim Sci.,* 29:309-314.

CURRICULUM VITAE

NAME - RICHARD BWALA

CONTACT DETAILS
AQUATIC ENVIRONMENT DIVISION,
NATURAL FISH FOOD PROGRAMME,
NATIONAL INSTITUTE FOR FRESHWATER FISHERIES
RESEARCH (NIFFR), P.M.B. 6006, NEW BUSSA,
NIGER STATE, NIGERIA.
PHONE: +234 9024462661. E-MAIL: richardl669@gmail.com

PERSONAL DATA

Date of birth	26th October 1973
Sex	Male
Religion	Christianity
Nationality	Nigerian
State of origin	Borno
Marital status	Married
Number of children	Two

EDUCATIONAL QUALIFICATIONS

M.Sc Applied Hydrobiology and Fisheries
(University of Jos, Nigeria) 2008

B.Sc Agriculture
(University of Maiduguri, Nigeria) 1998

Certificate in Microcomputer operation and management
(Quantum Computer Academy, Jos, Plateau State, Nigeria) 2001

WORK EXPERIENCE

National Youth Service Corp (NYSC).
Ogun State, Nigeria. 2000

National Institute for Freshwater Fisheries Research
(NIFFR), New Bussa, Niger State, Nigeria 2002 to date

DUTIES/RESPONSIBILITIES

My responsibilities are formulation and execution of research projects, compilation andinterpretation of research data, preparation of quarterly and annual reports on research projects, training and supervision of subordinate staff and tertiary students on Industrial work attachment.

WORKSHOP/CONFERENCES ATTENDED

International

Larvi conference, Ghent University, Belgium 2017

Start-to-Finish guide to research projects for Agri-Scientists, Organised by the African Doctoral Academy, Stellenbosch University, South Africa 2017

Oral Paper presentation on laboratory test for salinity and *Artemia* strain Selection for use in mass culture. Presented at the conference of Aquaculture Association of South Africa. 2015

Training on "Web 2.0 Tools for research support and networking in Africa" organized by Technical Centre for Agricultural and Rural Cooperation (CTA) *ACP-EC Cotonou Agreement*, in collaboration with Council for Science and Industrial Research- Institute for Scientific and Technological Information (CSIR – INSTI) Accra, Ghana. 2009

Local

Fisheries Society of Nigeria (FISON) conference Held at Federal University of Technology, Minna, Niger State, Nigeria 2011

Workshop on Biological Control of Invasive Weed Held in Benue and Kaduna States, Nigeria 2010

Fisheries Society of Nigeria (FISON) conference Held at ASCON, Badagry, Lagos State, Nigeria 2010

Fisheries Society of Nigeria (FISON) conference Held at Federal University of Technology, Akure, Ondo State, Nigeria 2009

Capacity building workshop on standard operations of Atomic Spectrometry (AAS) and Poly Acrylamide Gel Electrophoresis (PAGE) organized by NIFFR, New Bussa 2009

Fisheries Society of Nigeria (FISON) conference Held at Arewa House, Kaduna State, Nigeria 2008

Fisheries Society of Nigeria (FISON) conference held in Birnin Kebbi, Kebbi State, Nigeria 2007

Capacity building workshop on PowerPoint preparation and presentation organized by NIFFR, New Bussa	2007
Fisheries Society of Nigeria (FISON) conference held in Ilorin, Kwara State, Nigeria	2004
Workshop on Zooplankton Production/water Quality Management, organized by Winrock International: Farmer to Farmer programme at the NIFFR, New Bussa	2002

MEMBERSHIP OF PROFESSIONAL ORGANISATION

Memberof the Fisheries Society of Nigeria (FISON)

PUBLICATIONS

Thesis:

Bwala, R. 2008. The use of pig dung as fertilizer for the growth and survival of the Nile Tilapia (*Oreochromis niloticus*) in outdoor concrete tanks. M.Sc. Thesis in Hydrobiology and Fisheries, University of Jos, Nigeria.

Contribution in a book:

Bwala, R. 2010. A review of the physico-chemical characteristics of Lake Kainji, in Raji, A., Okaeme, A.N. & Ibeun, M.O. (eds.). Forty years on Lake Kainji Fisheries Research. A publication of National Institute for Freshwater Fisheries Research, New Bussa, Niger State, Nigeria. 144-150

Journal

Bwala, R., Salie, K. & Van Stappen, G. 2018. Ovoviviparously produced *Artemia* nauplii are a suitable live food source for the larvae of the African catfish (*Clarias gariepinus*: Burchell, 1822). *Aquaculture Research*, 49:3319-3328.

Bwala, R., Ovie, S.I., Ajayi, O. & Haruna, A. 2010. Preliminary study on the limnology and plankton abundance inrelation to fish production in some NIFFR reservoirs. *Report and Opinion Journal,* 2(6):9-15.

Bwala, R. & Omoregie, E. 2009. Organic enrichment of fish ponds: Application of pig dung vs. Tilapia yield. *Pakistan Journal of Nutrition,* 8(9):1373-1379.

Bwala, R., Ogunfowora, O.O., Uka, U.N. & Ifejika, P.I. 2009. Studies on primary productivity of ponds fertilized with pig dung at varying levels of concentration. *Best Journal,* 6(1): 111-115.

Bwala, R., Okaeme, A.N. & Raji, A. 2009. The status of organic fish farming in Nigeria: The way forward. *International Journal of Zoology,*1(1):20-24.

Ovie, S.I., **Bwala, R.** & Ajayi, O. 2011. A preliminary study on the limnological stock assessment, productivity and potential fish yield of Omi Dam, Nigeria. *African Journal of Environmental Science and Technology,* 5(11):956-963.

Ovie, S.I., & **Bwala, R.** 2012. Comparative analysis of the limnological stock assessment, productivity and potential fish yield of Ojirami and Oguta Lakes, Nigeria. *Journal of Tropical Freshwater Biology,* 21(1): 1-13

Bwala, R., Sule, A.M., Yem, I.Y., Rafiu, A. & Simeon, N. 2010. Indigenous fish identification methods in Lakes Kainji and Jebba, Nigeria. *Report and Opinion Journal,* 2(6):16-22.

Yem, I.Y., **Bwala, R.,** Bankole, N.O., Olowosegun, M.O. & Yaji, A. 2011. Analysis of Ichthyofaunal diversity and peculiarities of some Lakes in Nigeria. *Journal of Fisheries International,* 6(1):26-30.

Raji, A., Okaeme, A.N., Omorinkoba, W. & **Bwala, R.** 2012. Illegal fishing of inland water bodies in Nigeria: Kainji experience. *Continental J. Fisheries and Aquatic Science,* 6(1):47-58.

Ogunfowora, O.O., Yem, I.Y. & **Bwala, R.** 2009. Comparative study on the effect of fishing gears on fish quality in Kainji Lake Basin, Nigeria. *Best Journal,* 6(2):130-134.

Oluborode, G.B., Omorinkoba, W.S. & **Bwala, R.** 2010. Development and construction of an electric furnance and control system for fish drying. *Journal of Research in Agriculture,* 7(3):124-128.

Ogunfowora, O.O., Ojo, S.O., Uka, U.N. & **Bwala, R.** 2009. Reducing the incidence of by-catch through fishing gear modification: A review. *International Journal of Animal Science,*1(1):82-87.

Sule, A.M., Olowosegun, T., Sanni A.O. & **Bwala, R.** 2007. Socio economic variables affecting the adoption of solar tent technology for fish drying in Kainji Lake Basin. *Journal of Arid Zone Fisheries*, 3(2):45-53.

COMMUNITY WORK

Team Member: World Fish Centre/CGIAR/NIFFR International collaboration on participatory diagnosis and adaptive management of small-scale fisheries in the Niger River Basin, project number 72, CGIAR challenge programme on water and food (2009)

HOBBIES

Reading, travelling and meeting people.